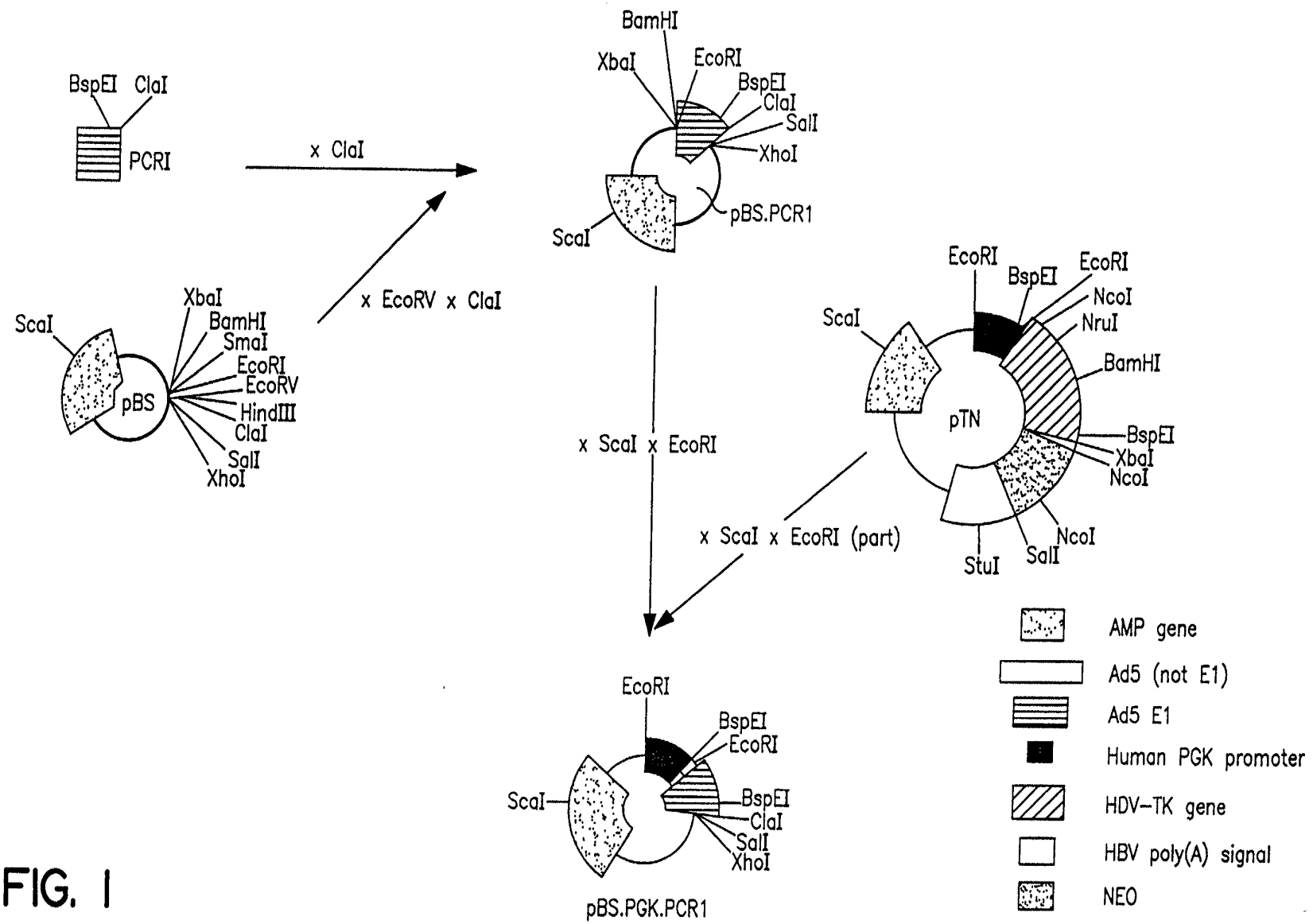


FIG. 1

Construction of pIG.E1a.E1b.X

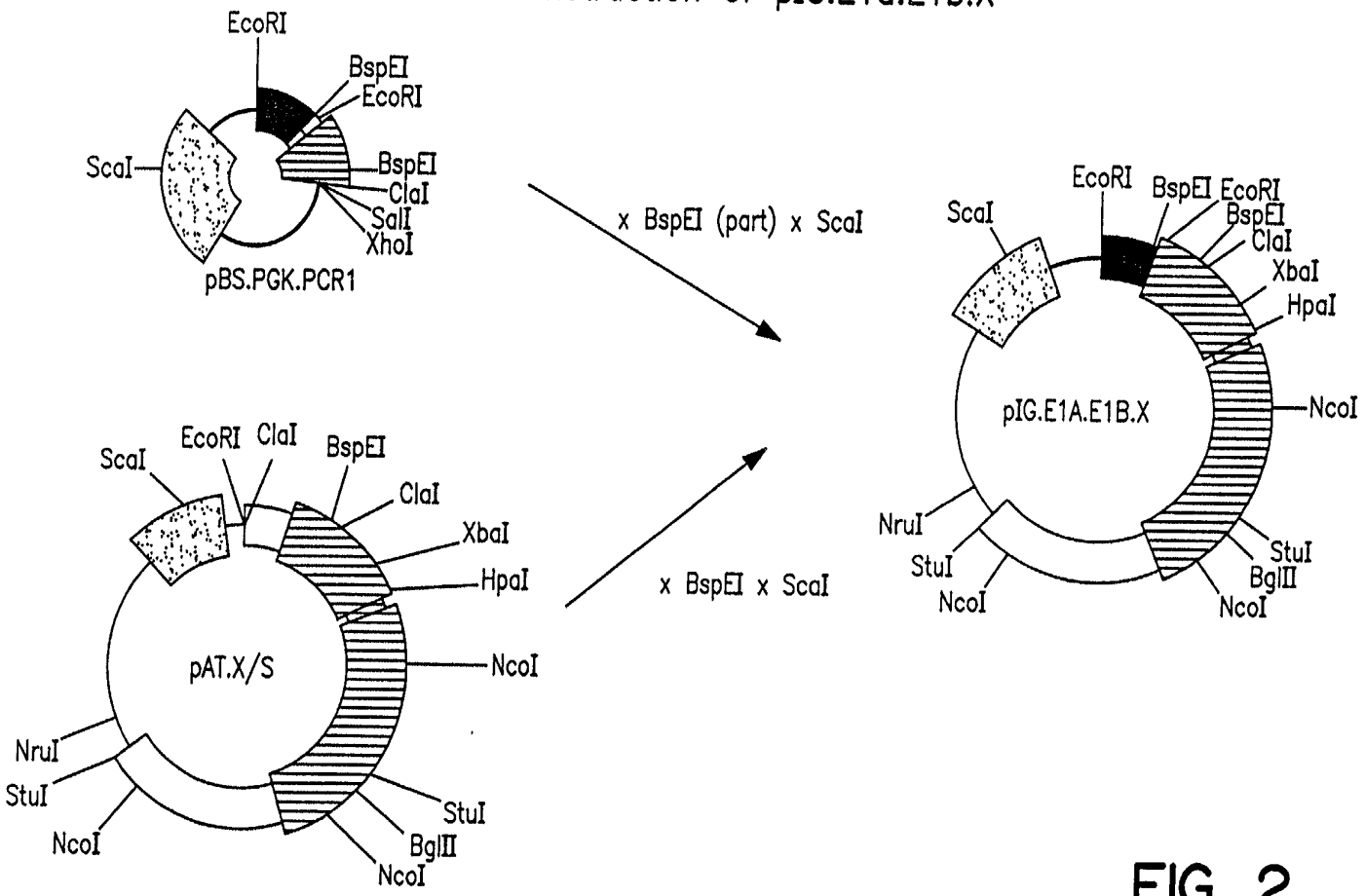


FIG. 2

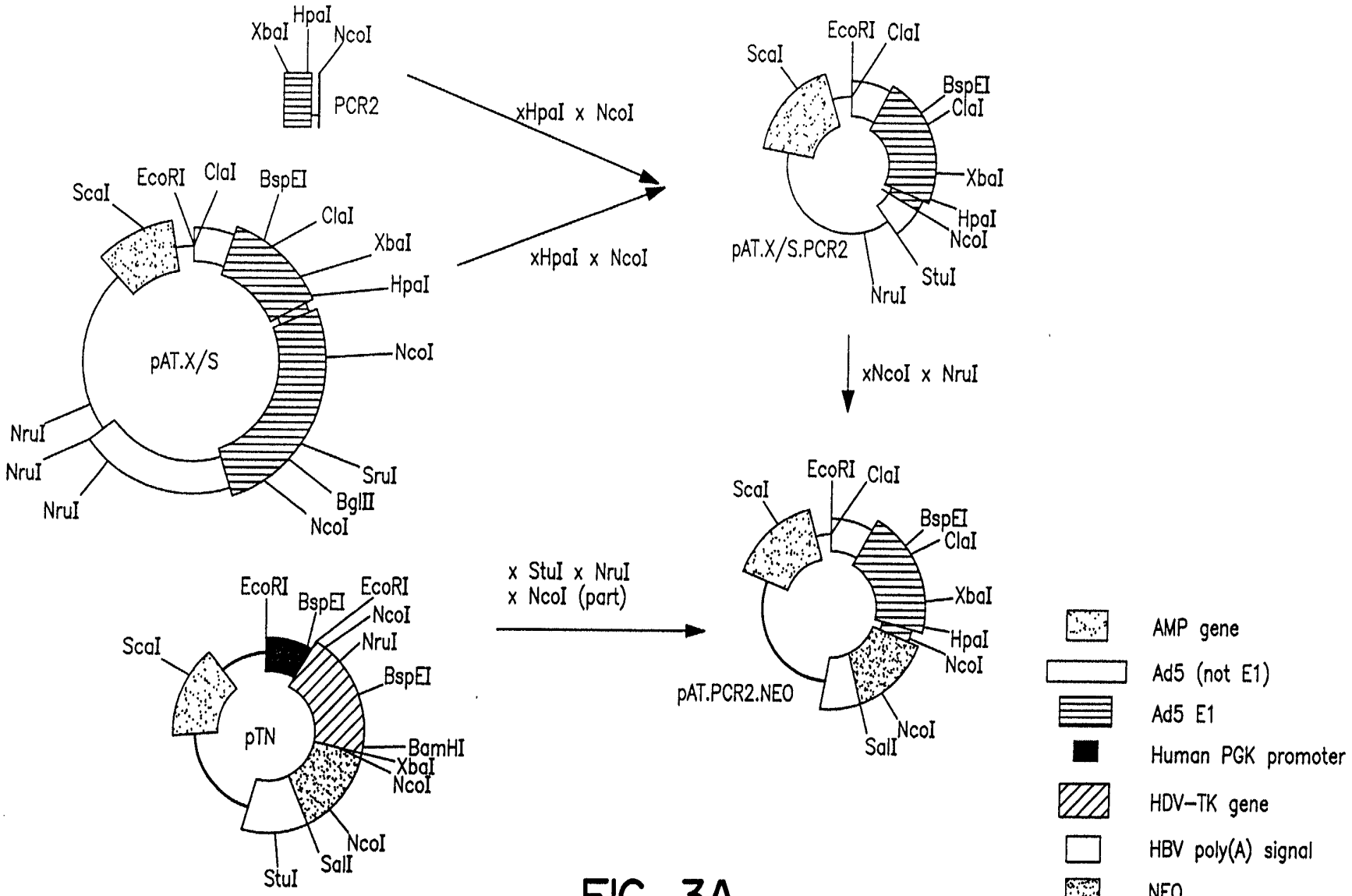

HpaI
XbaI
NcoI
PCR2
xHpaI x NcoI
xHpaI x NcoI
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.X/S
NruI
NruI
NruI
SruI
BglII
NcoI
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.X/S.PCR2
NruI
StuI
xNcoI x NruI
x StuI x NruI
x NcoI (part)
EcoRI
BspEI
EcoRI
NcoI
NruI
ScaI
BspEI
pTN
BamHI
XbaI
NcoI
NcoI
StuI
SalI
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.PCR2.NEO
NcoI
SalI
AMP gene
Ad5 (not E1)
Ad5 E1
Human PGK promoter
HDV–TK gene
HBV poly(A) signal
NEO

FIG. 3A

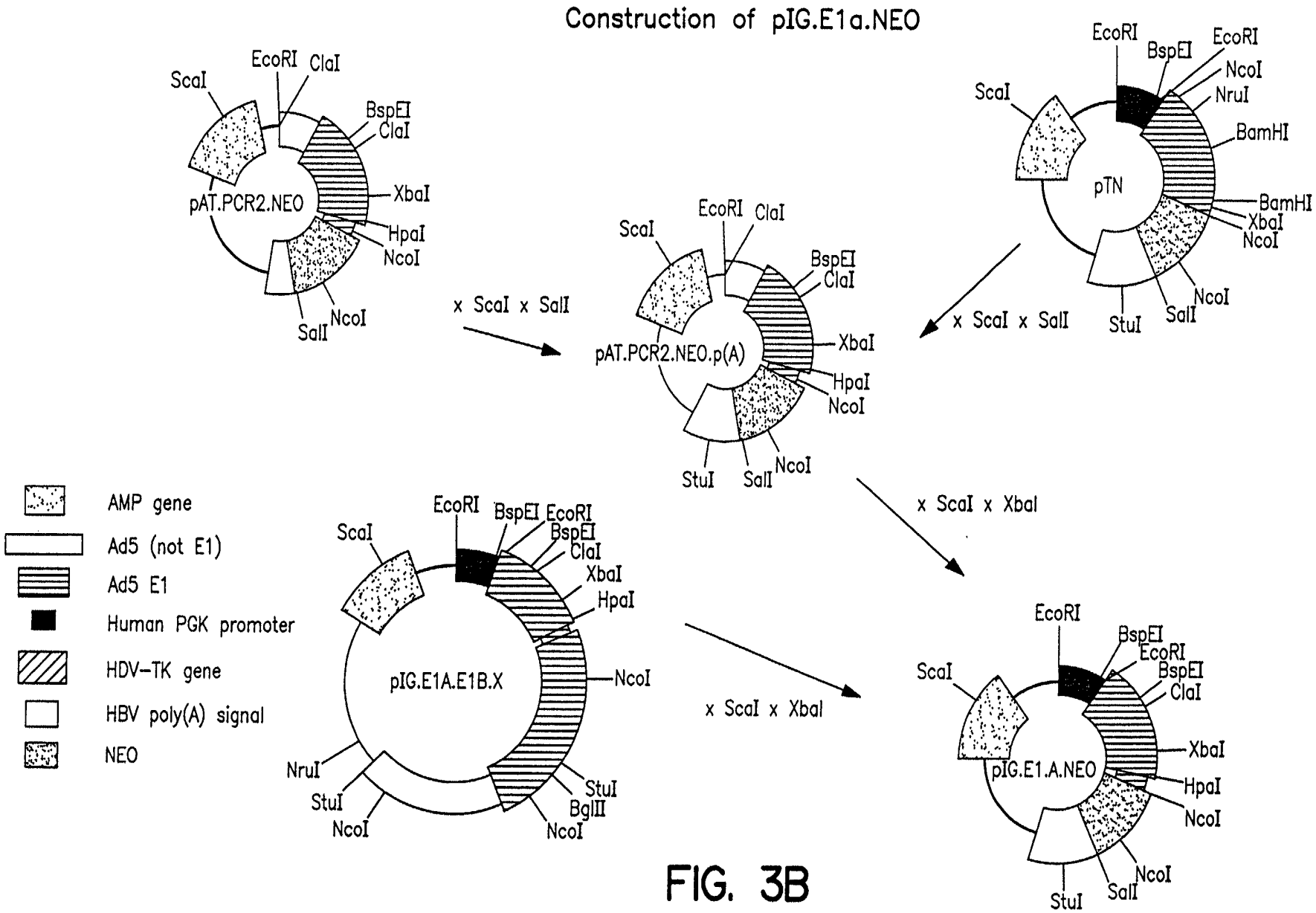
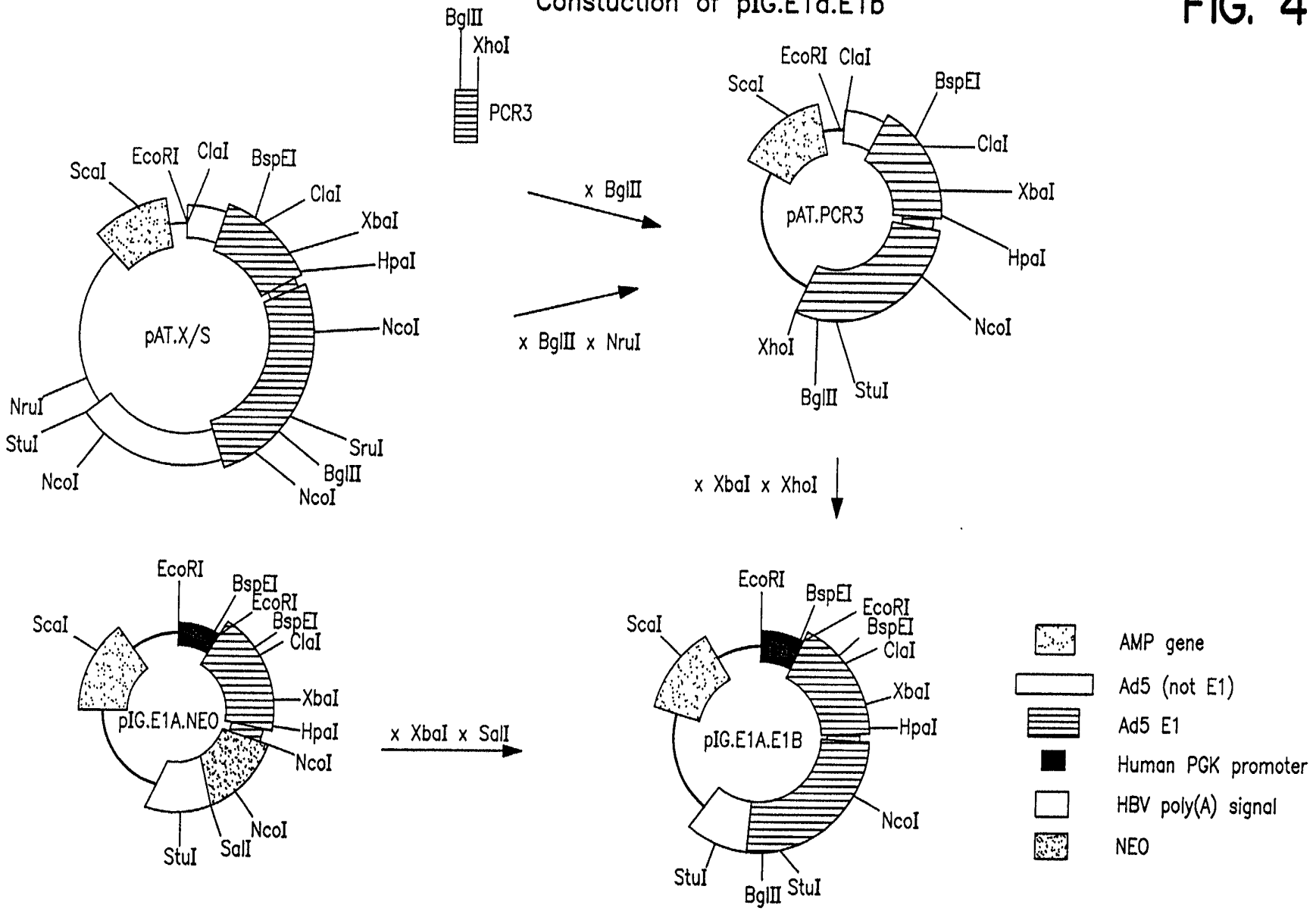

Construction of pIG.E1a.NEO
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
NcoI
SalI
pAT.PCR2.NEO
x ScaI x SalI
pAT.PCR2.NEO.p(A)
StuI
SalI
NcoI
x ScaI x SalI
pTN
EcoRI
BspEI
EcoRI
NcoI
NruI
BamHI
BamHI
XbaI
NcoI
NcoI
StuI
SalI
x ScaI x XbaI
pIG.E1A.E1B.X
EcoRI
BspEI
EcoRI
BspEI
ClaI
XbaI
HpaI
NcoI
NruI
StuI
NcoI
StuI
BglII
NcoI
x ScaI x XbaI
pIG.E1.A.NEO
EcoRI
BspEI
EcoRI
BspEI
ClaI
XbaI
HpaI
NcoI
NcoI
StuI
SalI
AMP gene
Ad5 (not E1)
Ad5 E1
Human PGK promoter
HDV-TK gene
HBV poly(A) signal
NEO

FIG. 3B

Constuction of pIG.E1a.E1b

FIG. 4



Construction of pIG.NEO

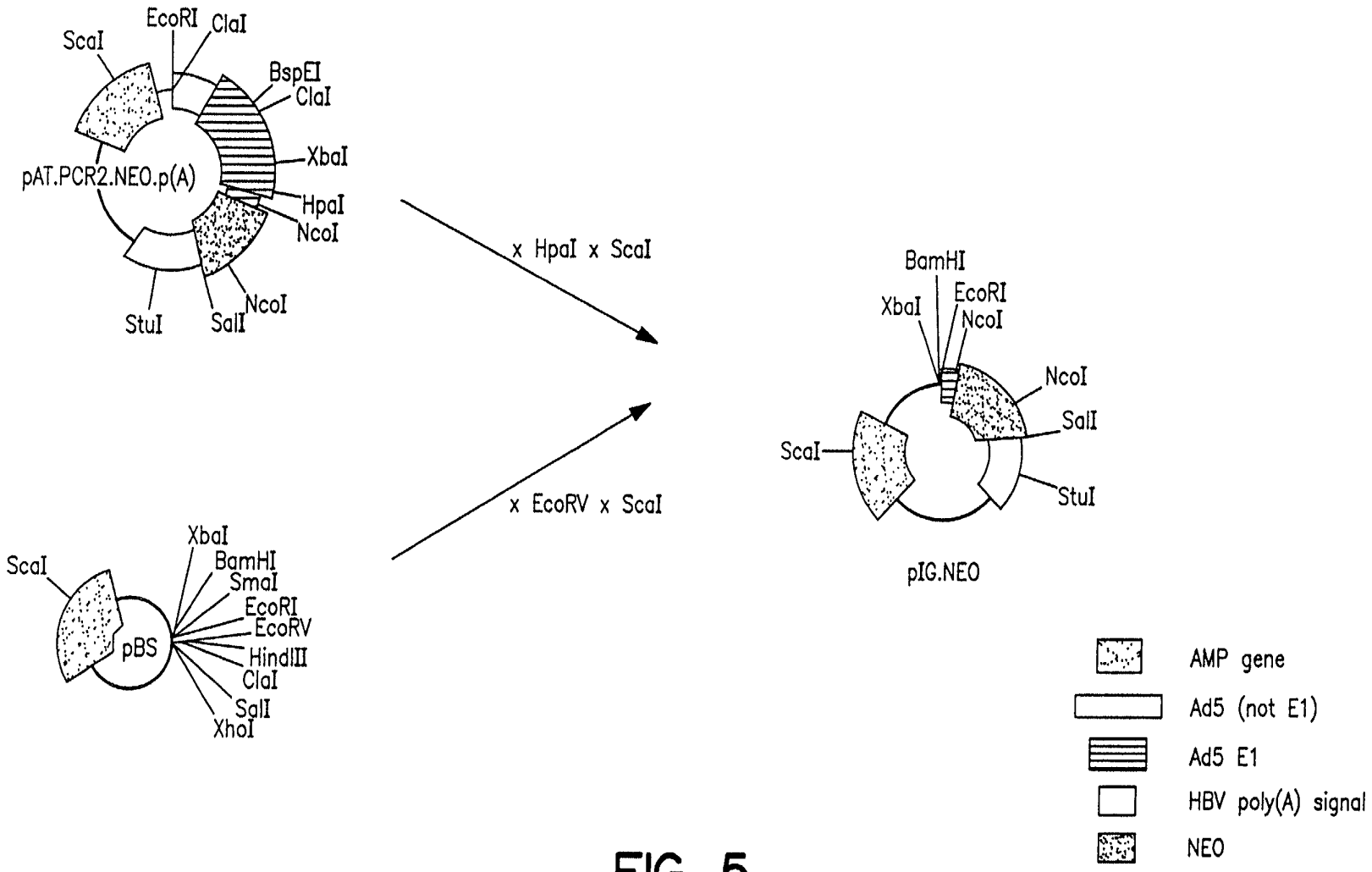
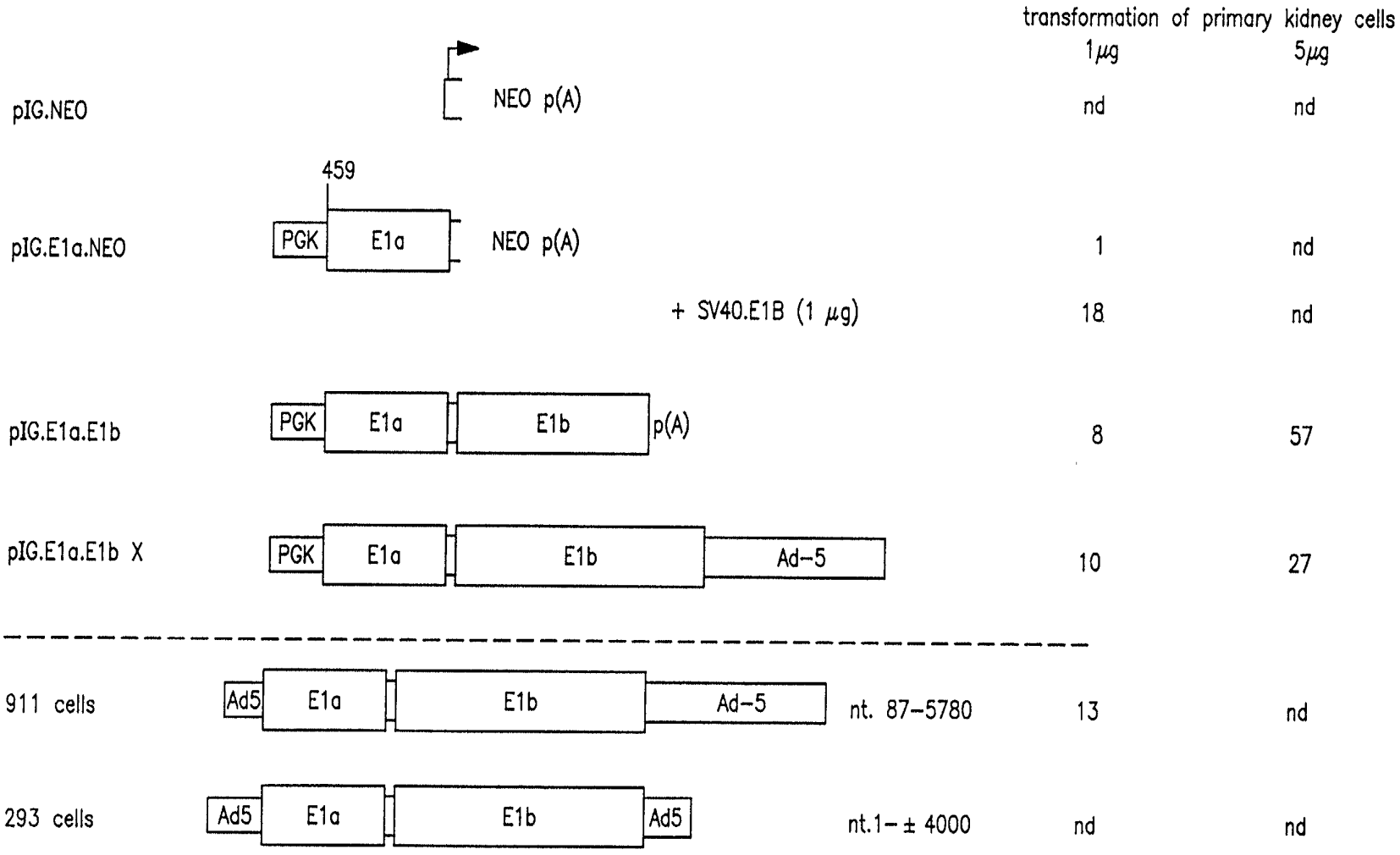


FIG. 5

Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



*average of 5 plates 21 days after transelection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones
(HER cells transfected with pIG.E1A.E1B)

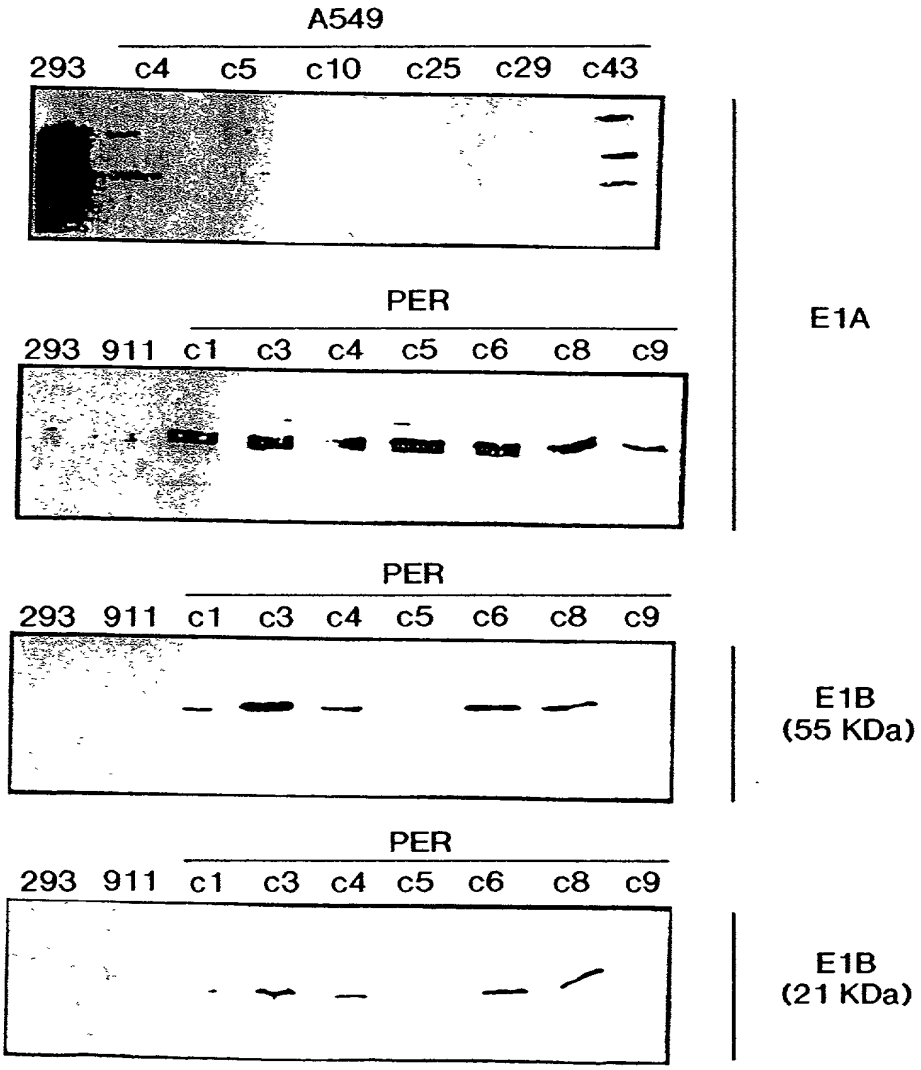


FIG. 7

Southern blot analyses of 293, 911 and PER cell lines

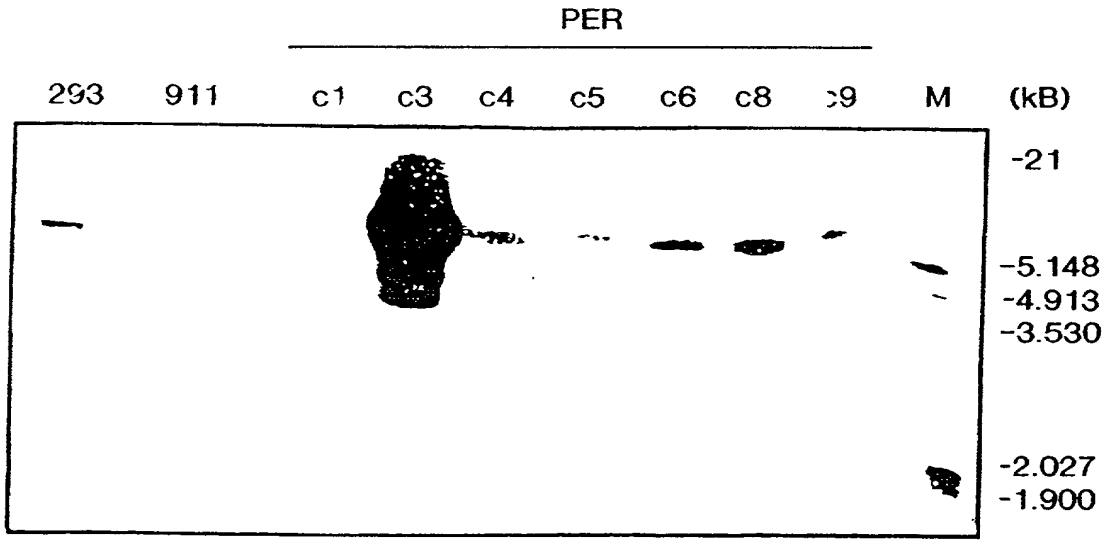


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5 µg pRSV.lasZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

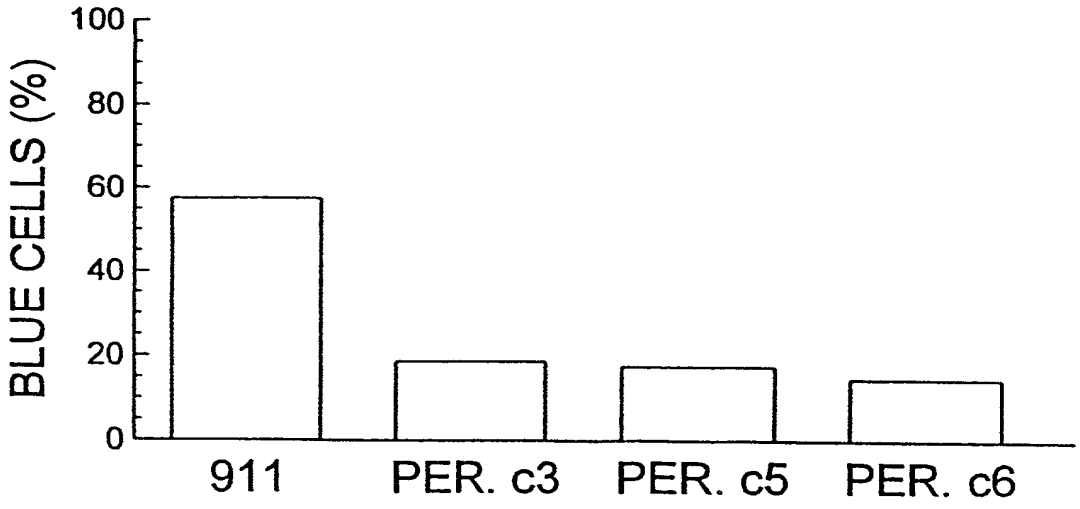


FIG. 9

Construction of pMLP1.TK

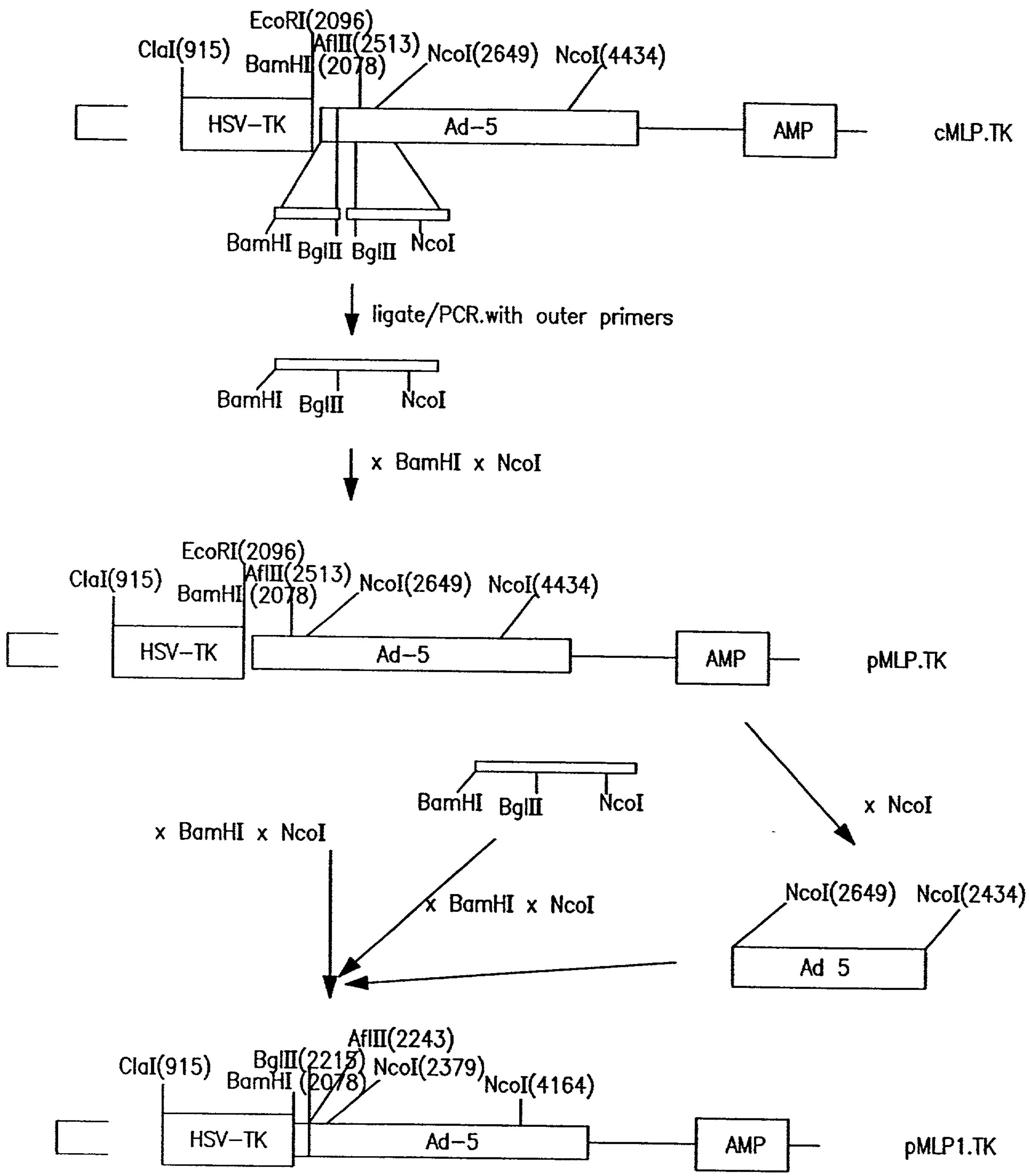


FIG. 10

New recombinant adenoviruses and packaging constructs without sequence overlap

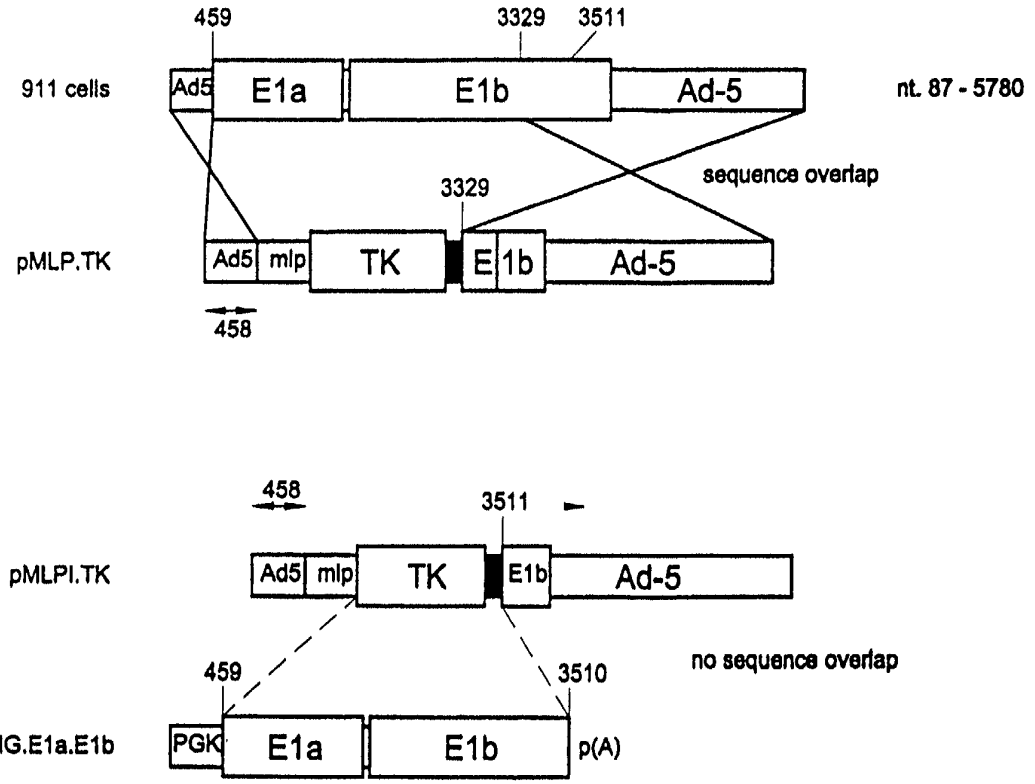
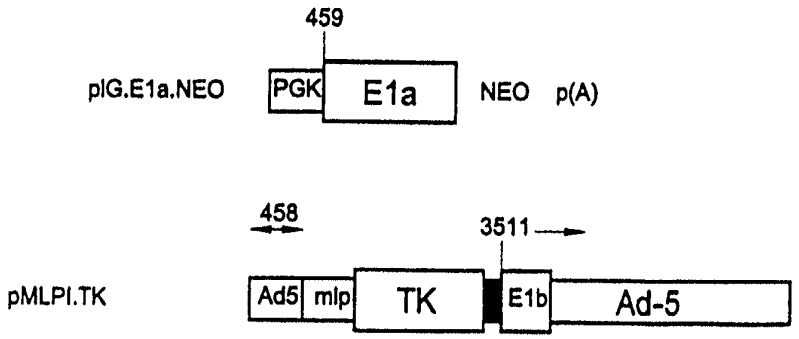


FIG. 1 IA

Packaging system based on primary cells

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418

FIG. 1 IB

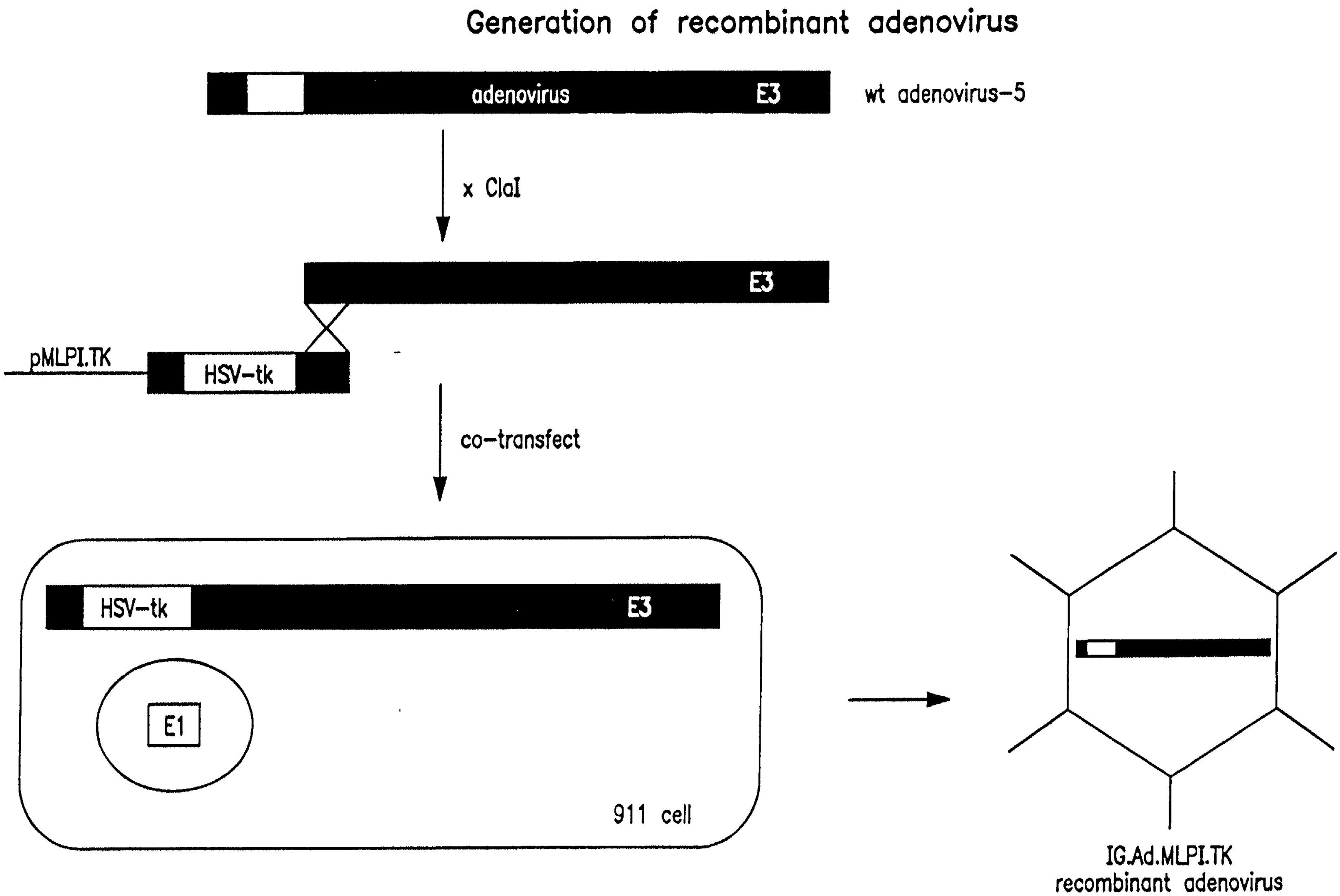

Generation of recombinant adenovirus
adenovirus
E3
wt adenovirus-5
x ClaI
E3
pMLPI.TK
HSV-tk
co-transfect
HSV-tk
E3
E1
911 cell
IG.Ad.MLPI.TK
recombinant adenovirus

FIG. 12

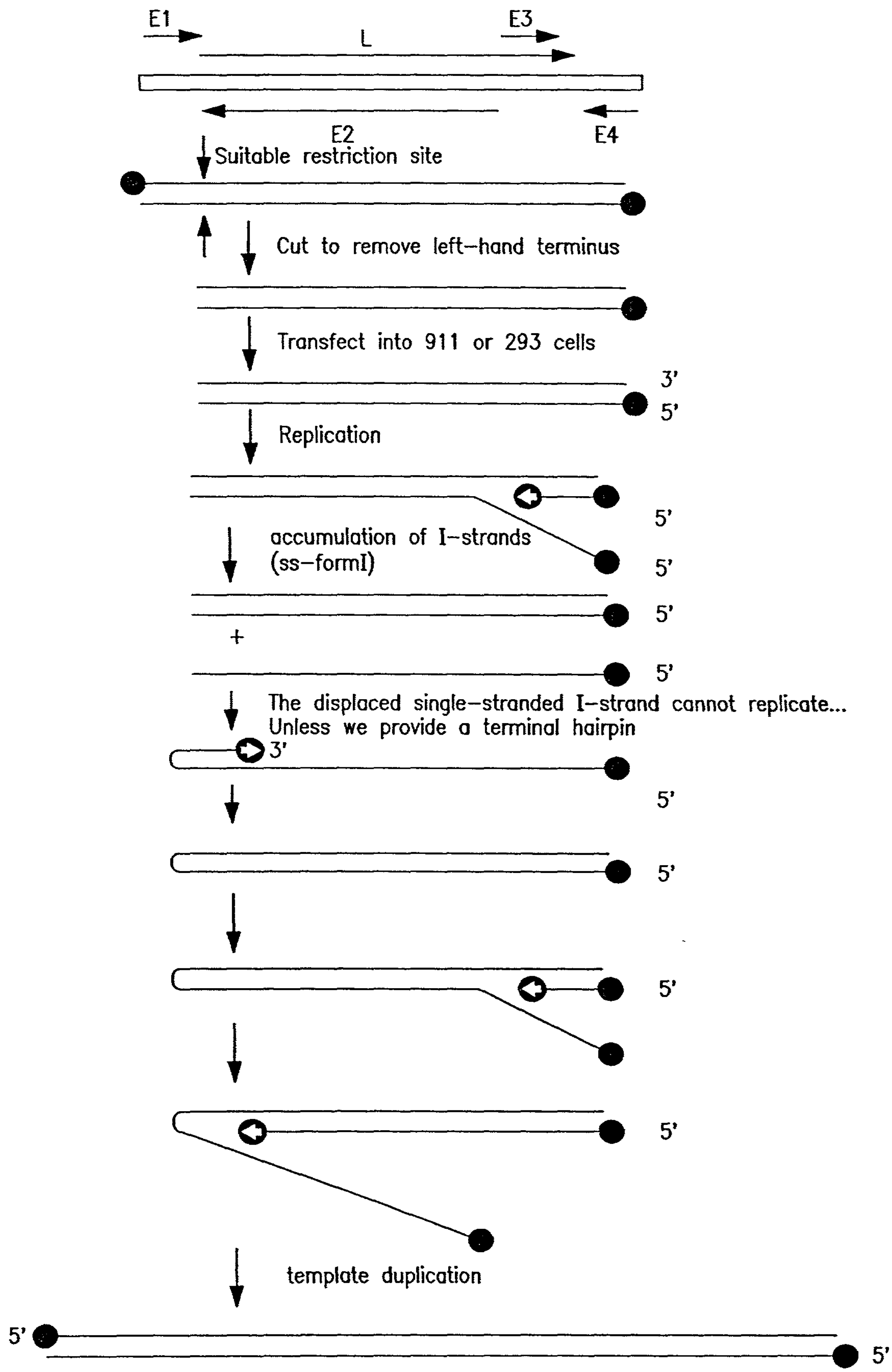

E1
E3
L
E2
E4
Suitable restriction site
Cut to remove left–hand terminus
Transfect into 911 or 293 cells
3'
5'
Replication
5'
accumulation of I–strands
(ss–formI)
5'
5'
+
5'
The displaced single–stranded I–strand cannot replicate...
Unless we provide a terminal hairpin
3'
5'
5'
5'
5'
template duplication
5'
5'

FIG. 13

Replication of Adenovirus

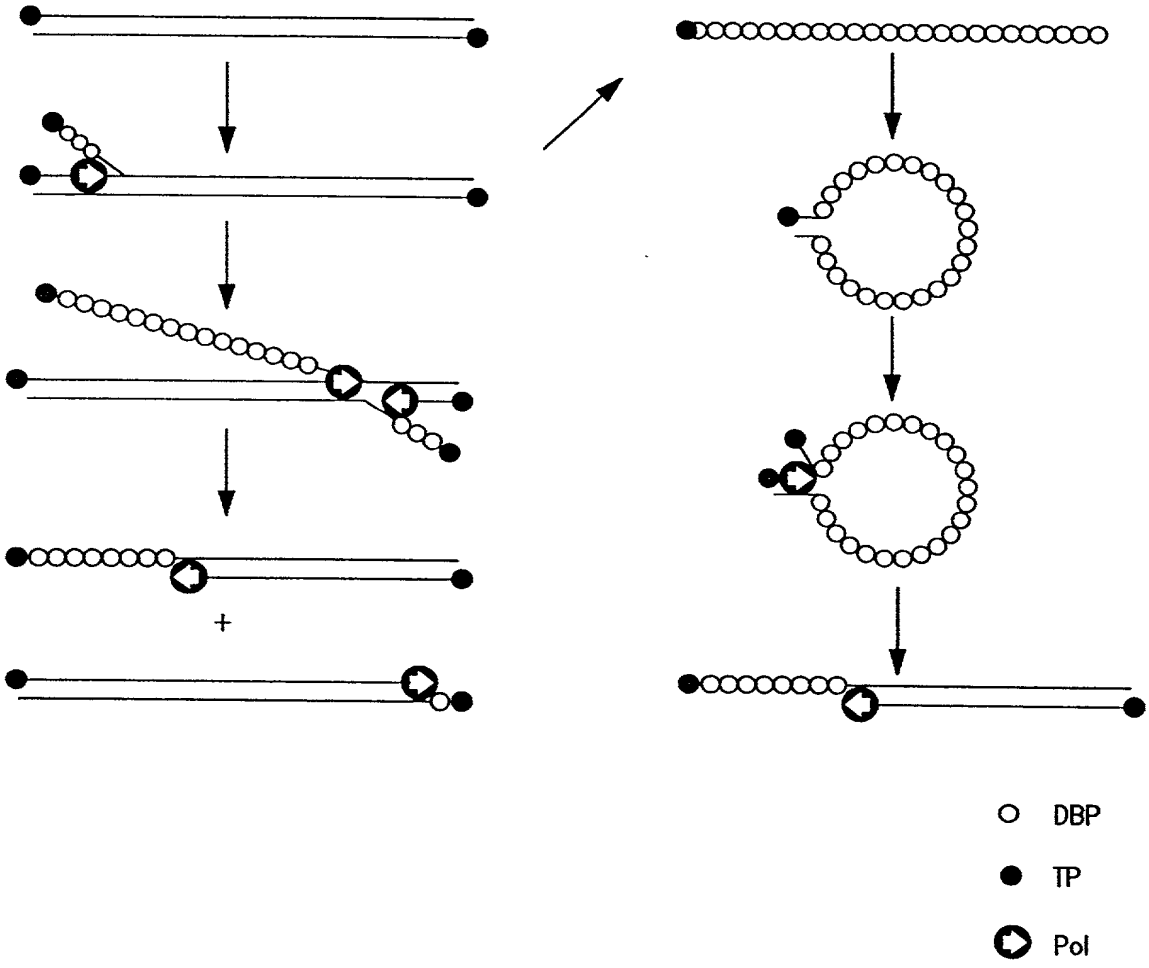


FIG. 14

The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.


```
5'-GTACACTGACCTAGTGCCGCCCGGGCA
             ||||||||||||||||  A
          3'-GATCACGGCGGGCCCGA
```

FIG. 15

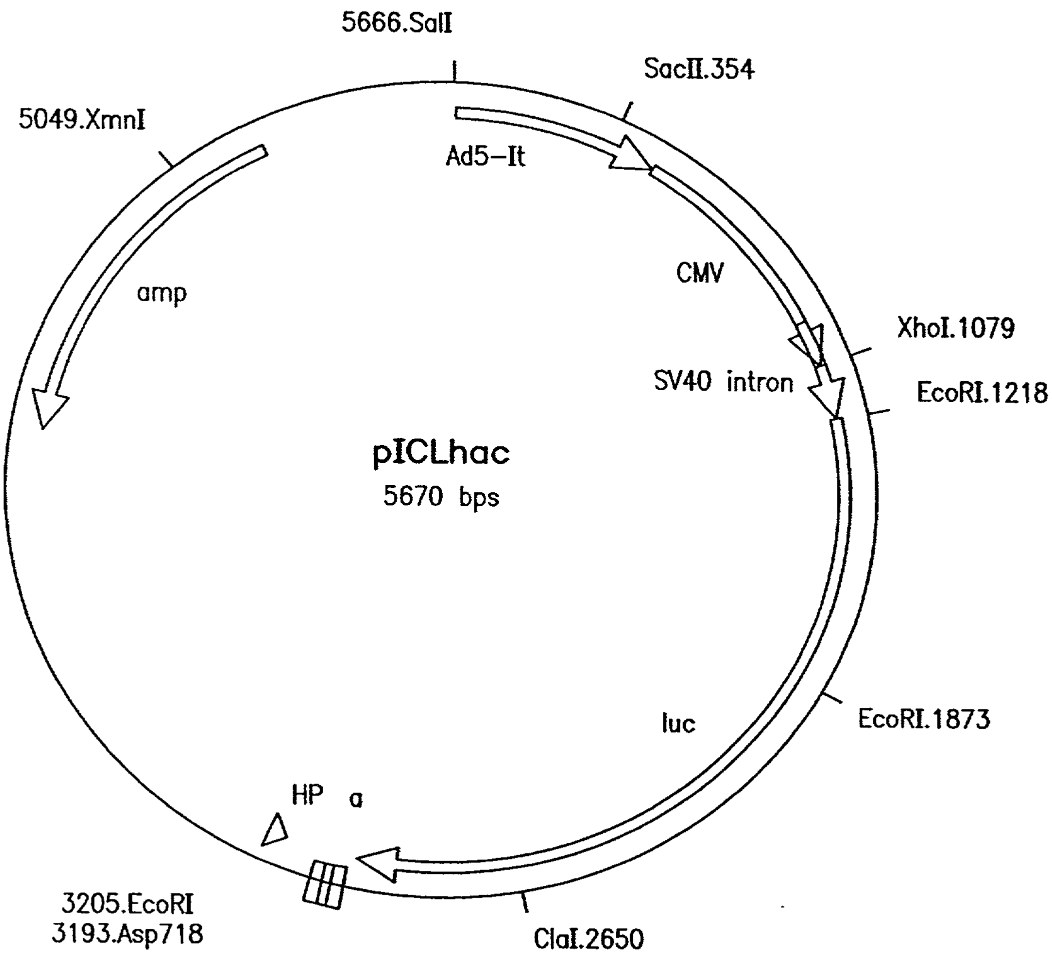

5666.SalI
SacII.354
5049.XmnI
Ad5-It
CMV
amp
XhoI.1079
SV40 intron
EcoRI.1218
pICLhac
5670 bps
luc
EcoRI.1873
HP
a
3205.EcoRI
3193.Asp718
ClaI.2650

FIG. 16

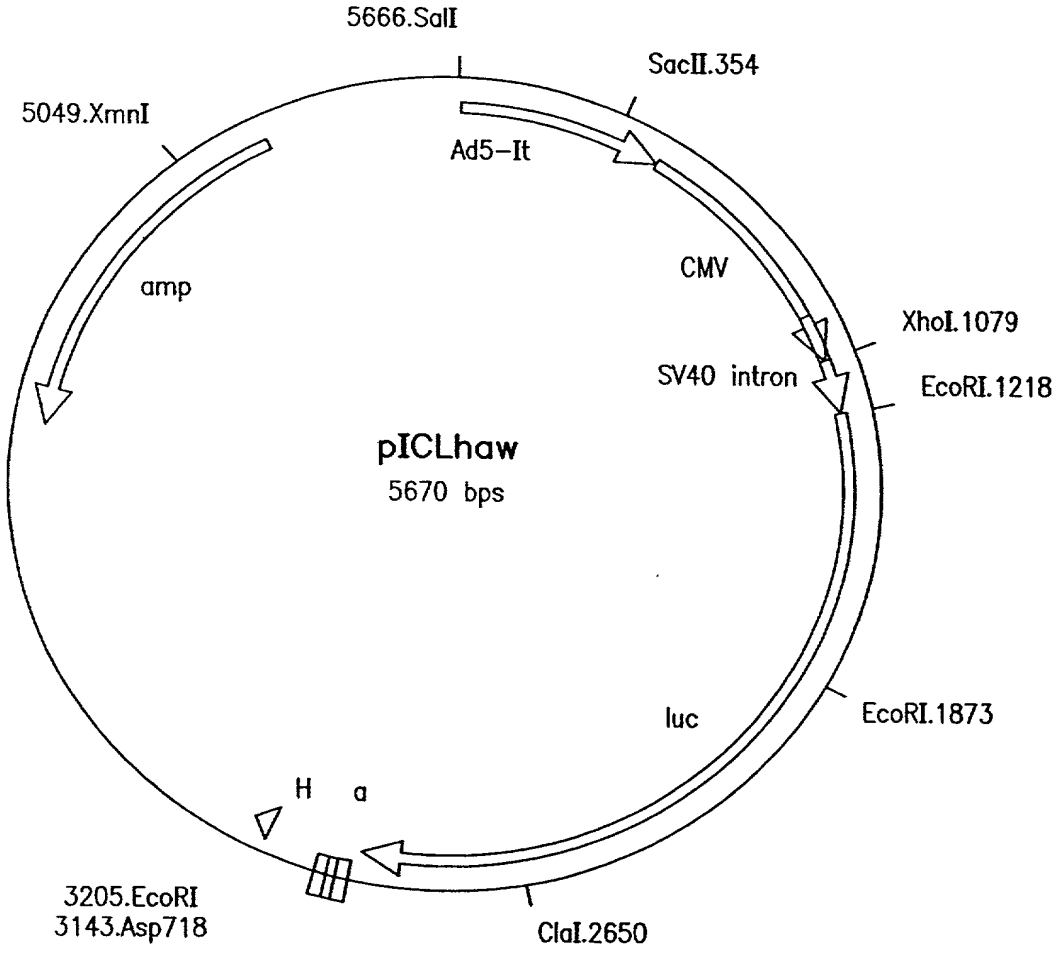

5666.SalI
SacII.354
5049.XmnI
Ad5-It
amp
CMV
XhoI.1079
SV40 intron
EcoRI.1218
pICLhaw
5670 bps
luc
EcoRI.1873
H
a
3205.EcoRI
3143.Asp718
ClaI.2650

FIG. 17

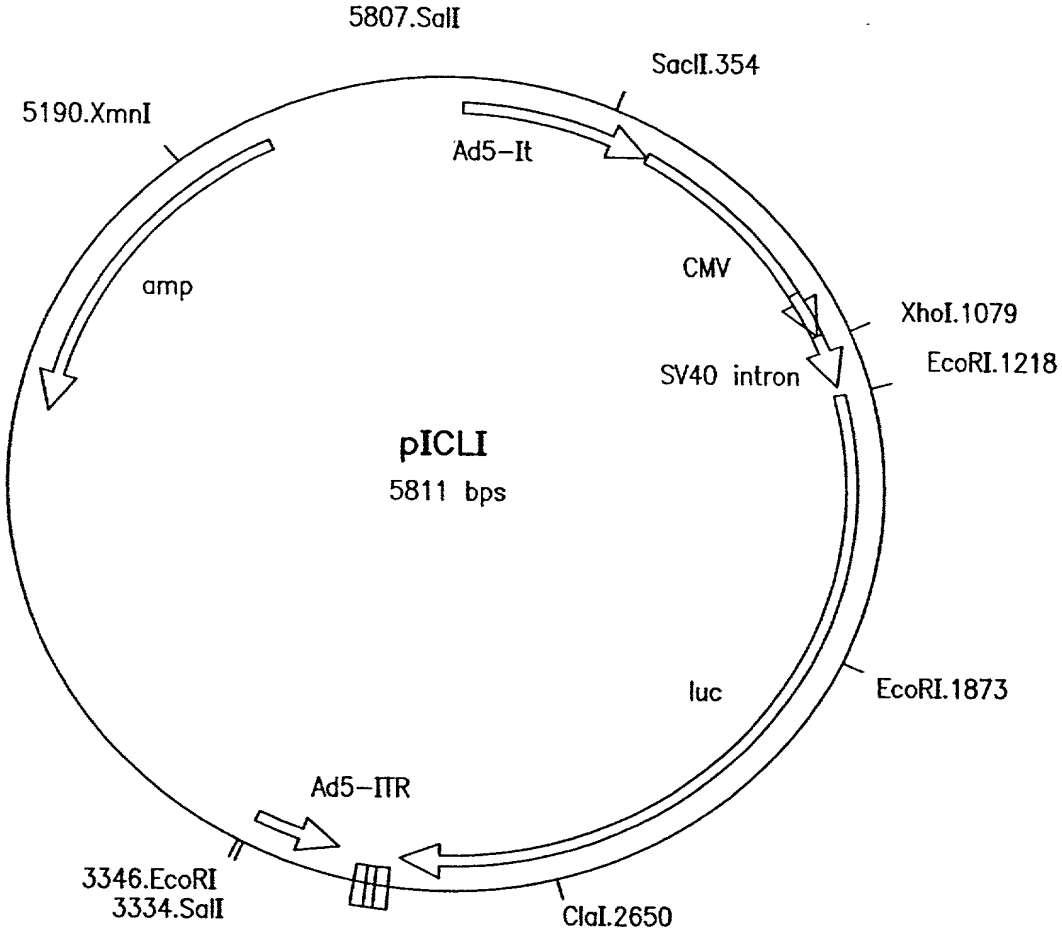

5807.SalI
SacII.354
5190.XmnI
Ad5-It
amp
CMV
XhoI.1079
EcoRI.1218
SV40 intron
pICLI
5811 bps
luc
EcoRI.1873
Ad5-ITR
3346.EcoRI
3334.SalI
ClaI.2650

FIG. 18

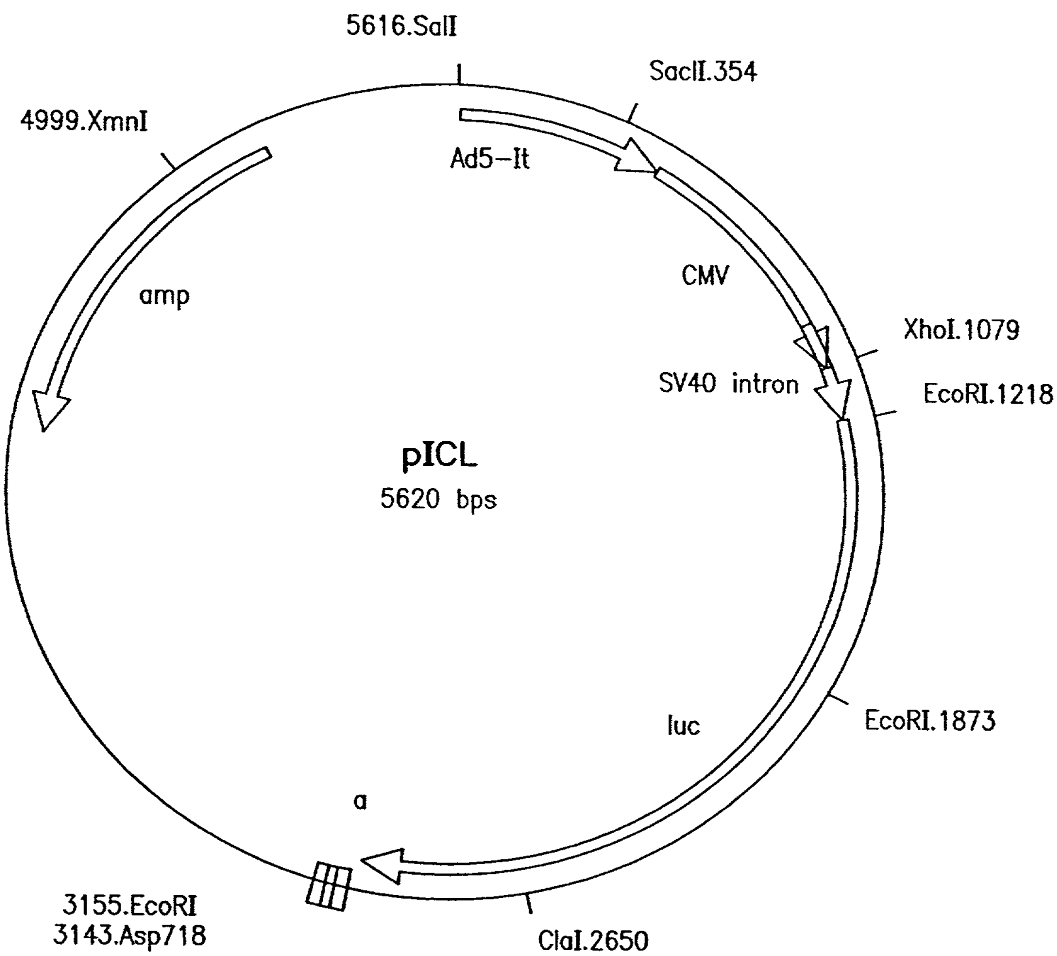

5616.SalI
SacII.354
4999.XmnI
Ad5-It
amp
CMV
XhoI.1079
SV40 intron
EcoRI.1218
pICL
5620 bps
luc
EcoRI.1873
a
3155.EcoRI
3143.Asp718
ClaI.2650

FIG. 19

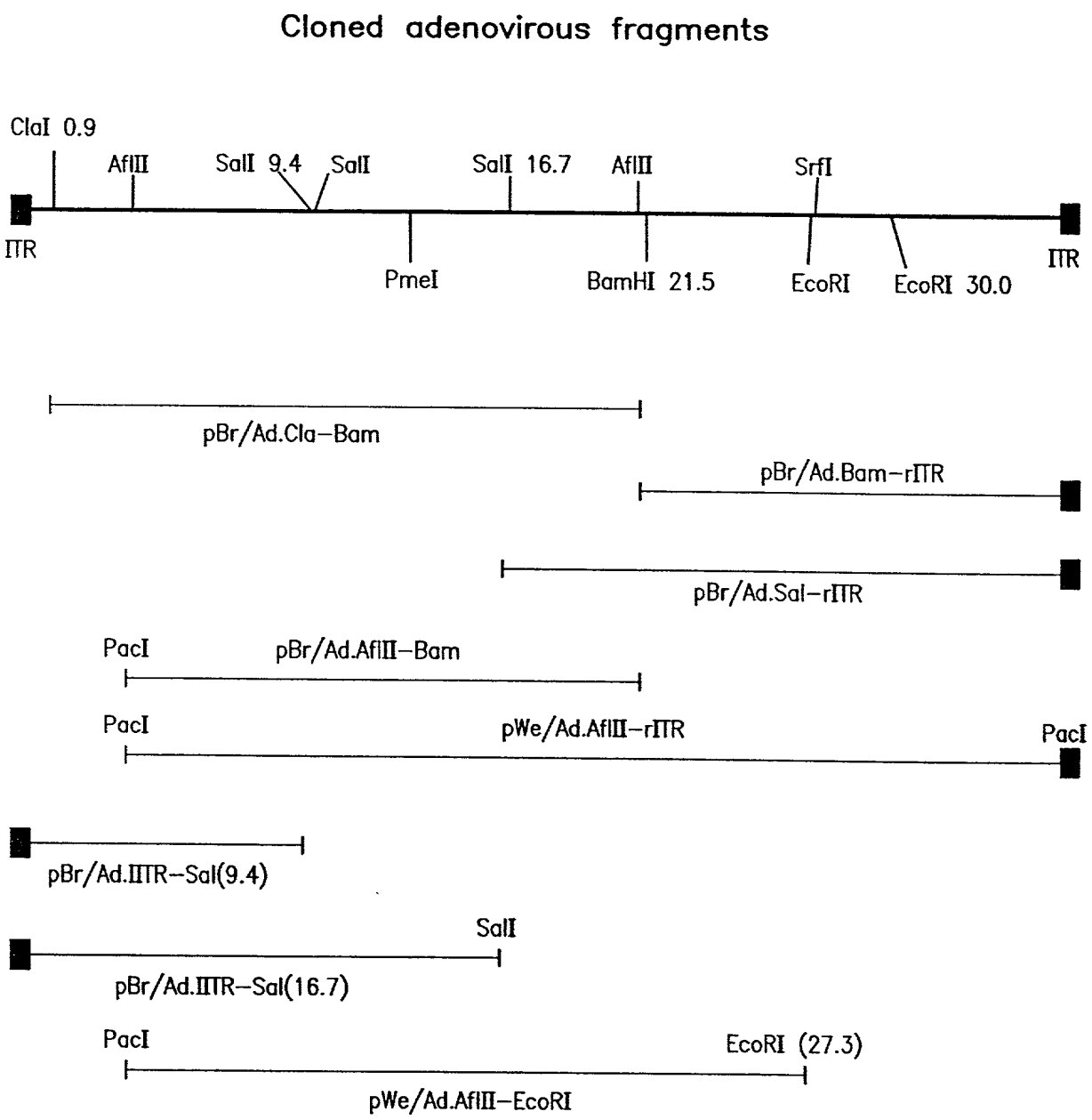


FIG. 20

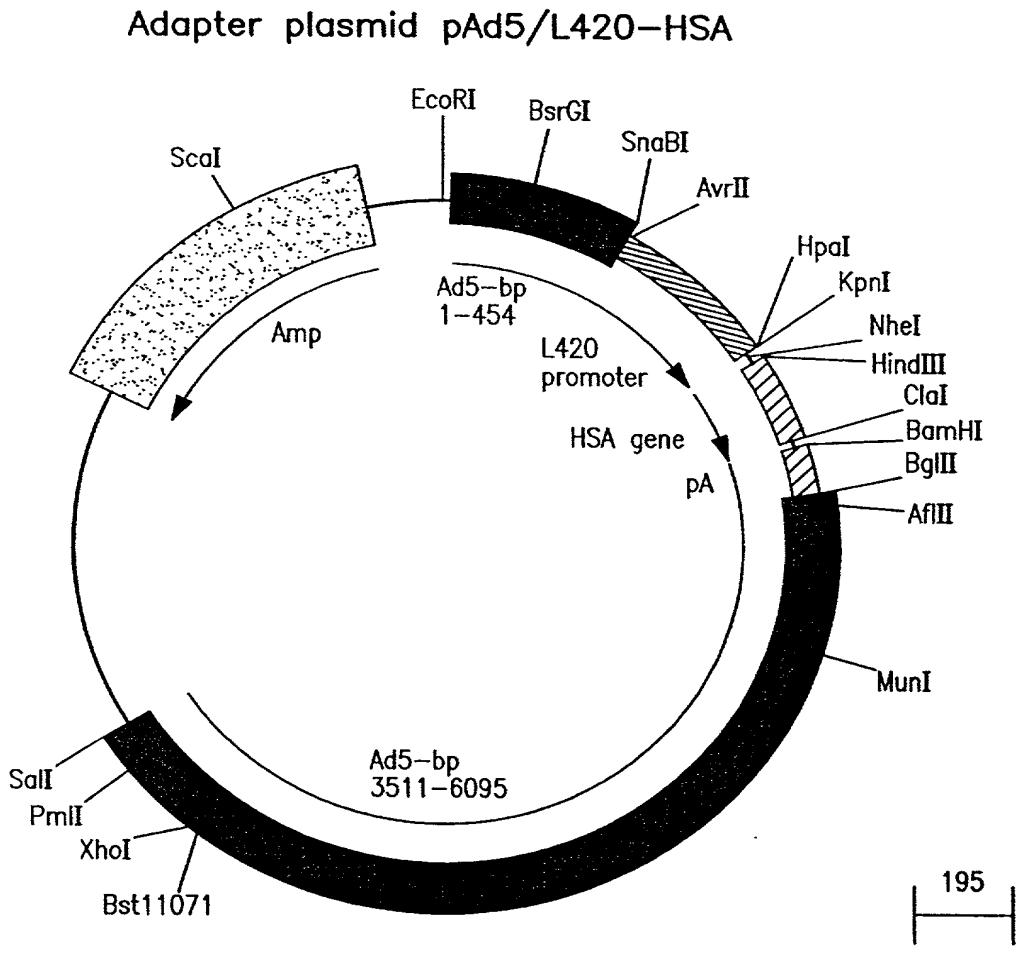


FIG. 21

Adapter plasmid pAd5/CLIP

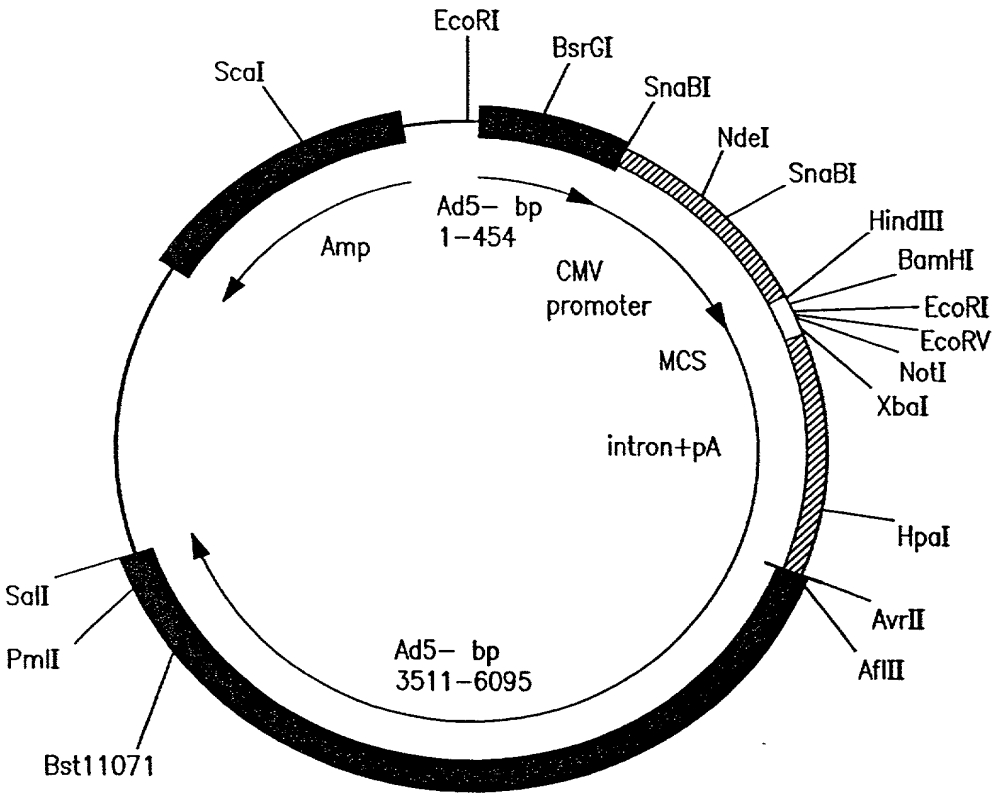


FIG. 22

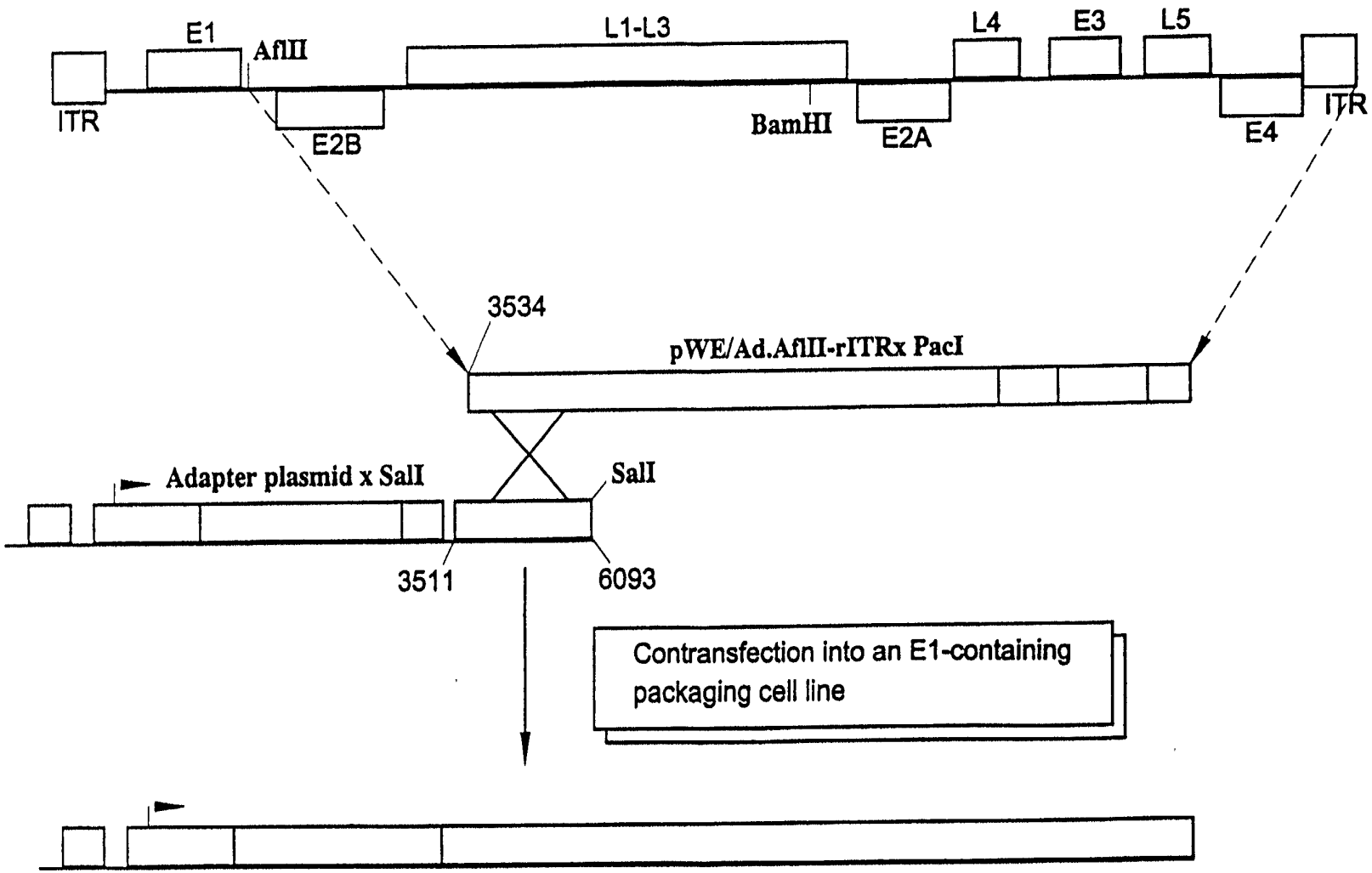


FIG. 23

Minimal adenovirus vector pMV/L420H

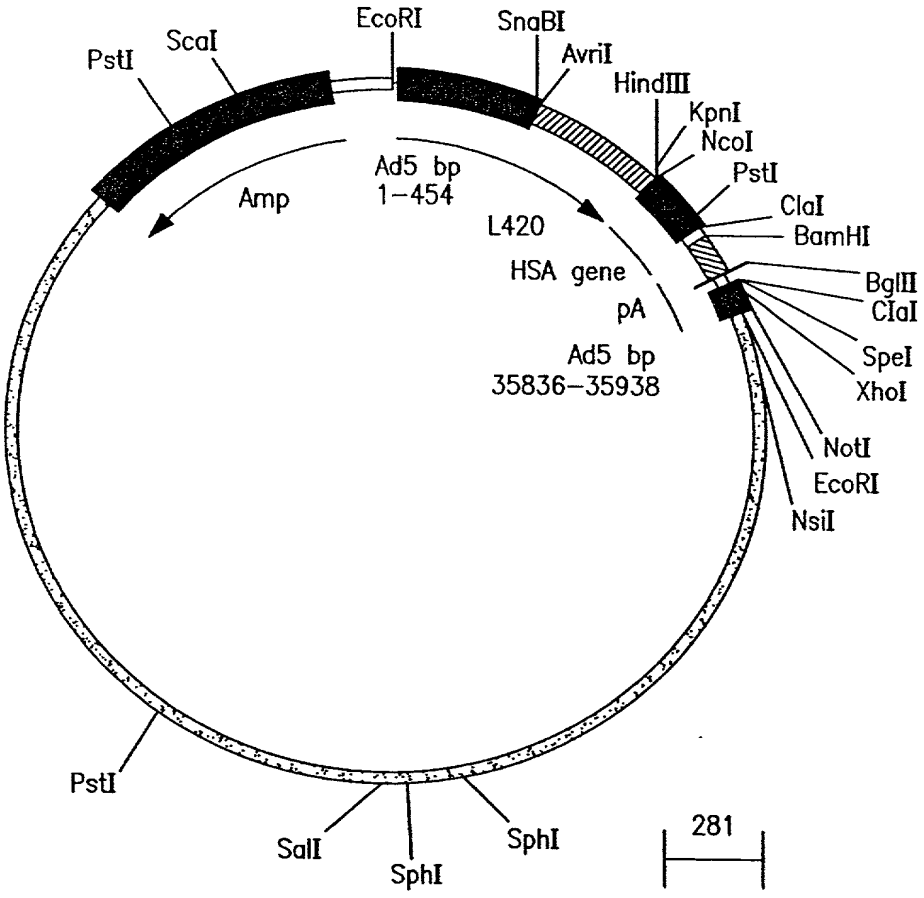


FIG. 24

Construction of pWE/AdΔ5'

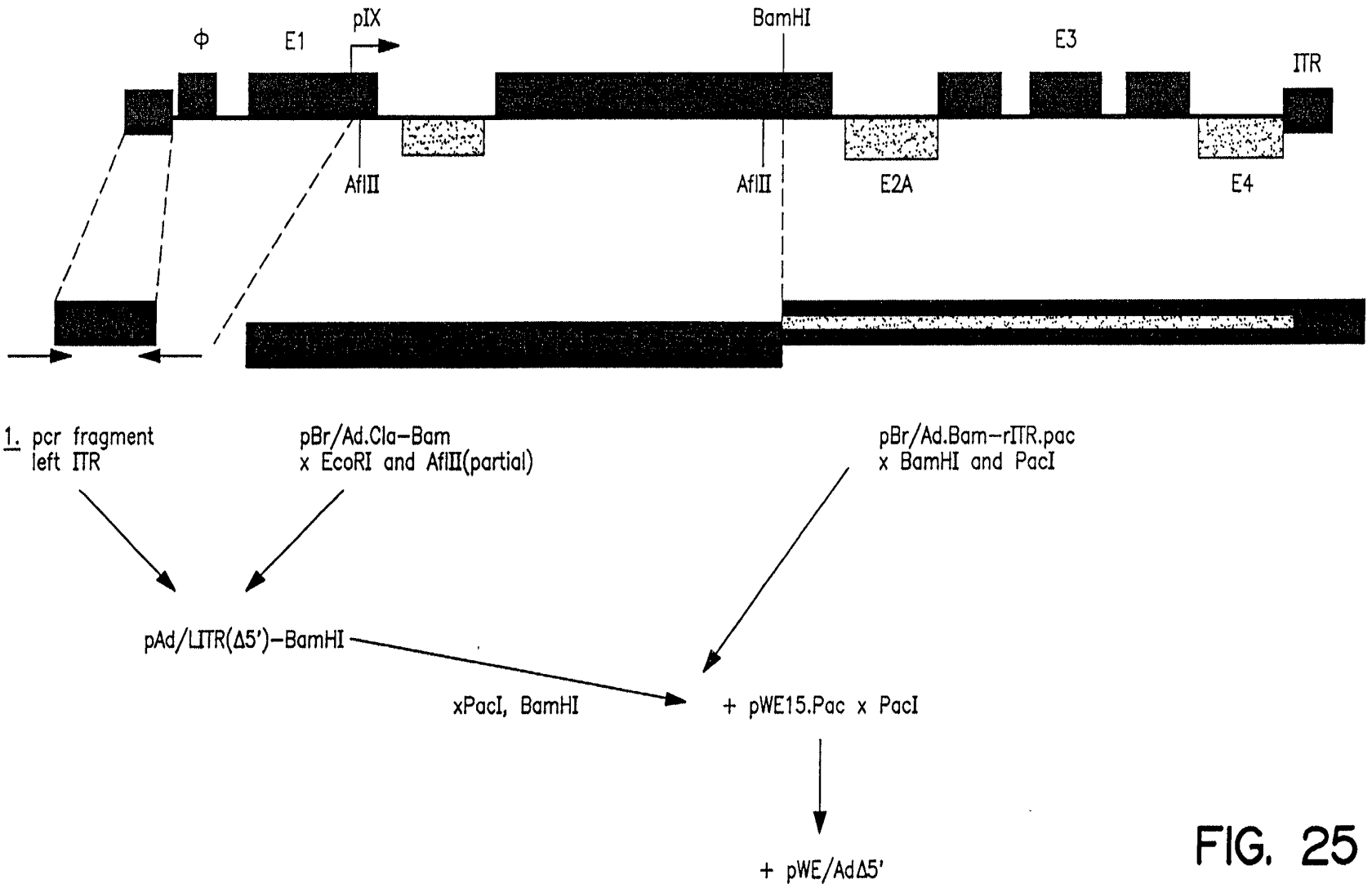


FIG. 25

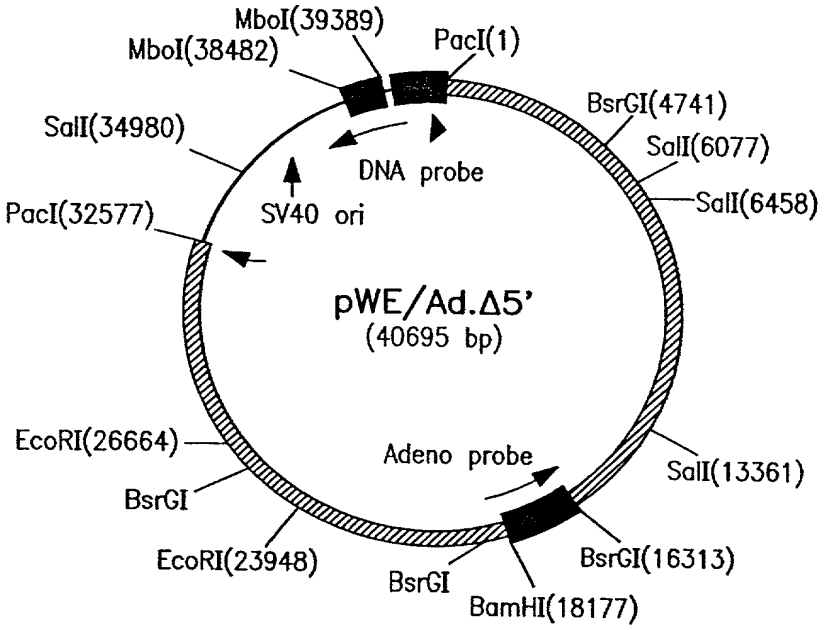

MboI(39389)
MboI(38482)
PacI(1)
BsrGI(4741)
SalI(6077)
SalI(6458)
SalI(34980)
DNA probe
SV40 ori
PacI(32577)
pWE/Ad.Δ5'
(40695 bp)
EcoRI(26664)
Adeno probe
SalI(13361)
BsrGI
BsrGI(16313)
EcoRI(23948)
BsrGI
BamHI(18177)

FIG. 26A

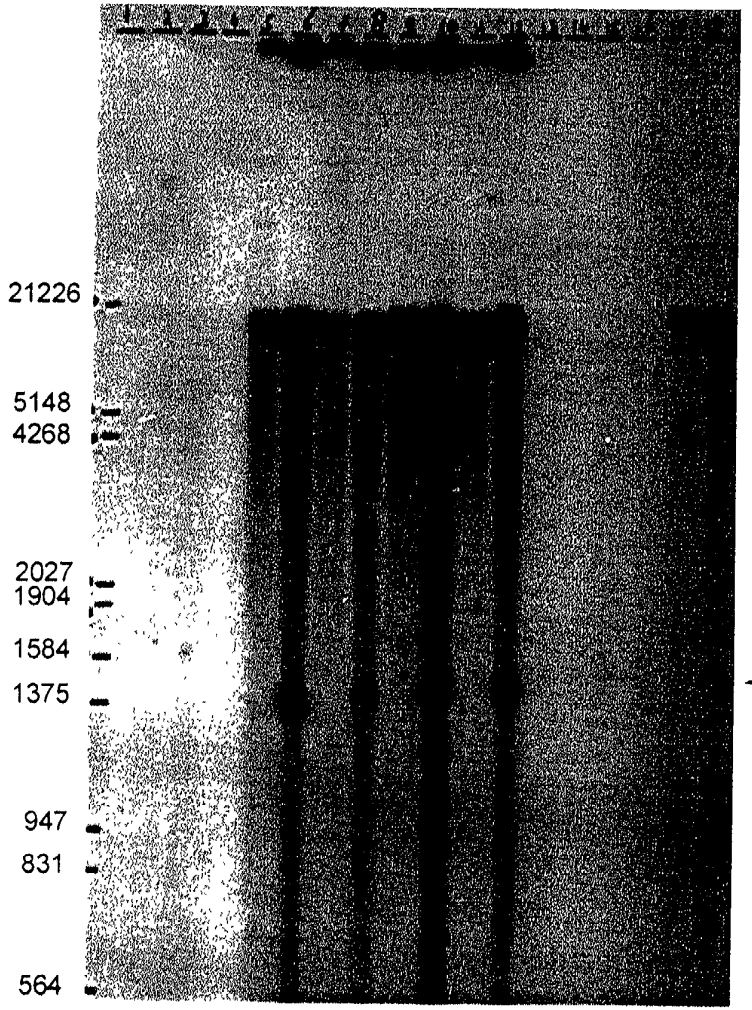


FIG. 26B

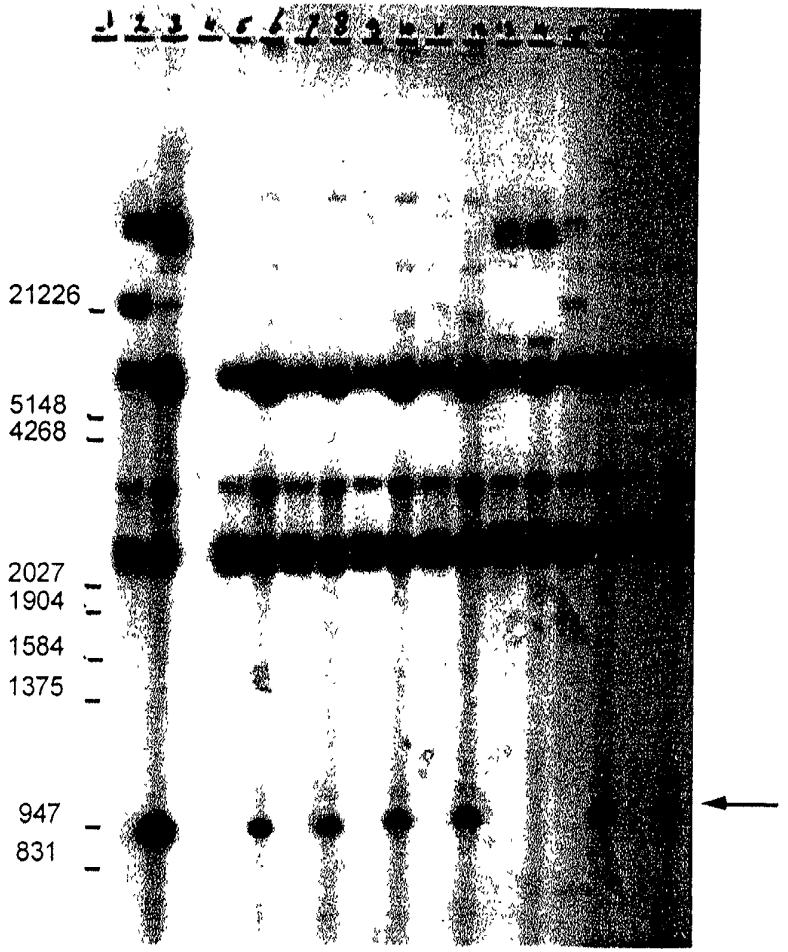
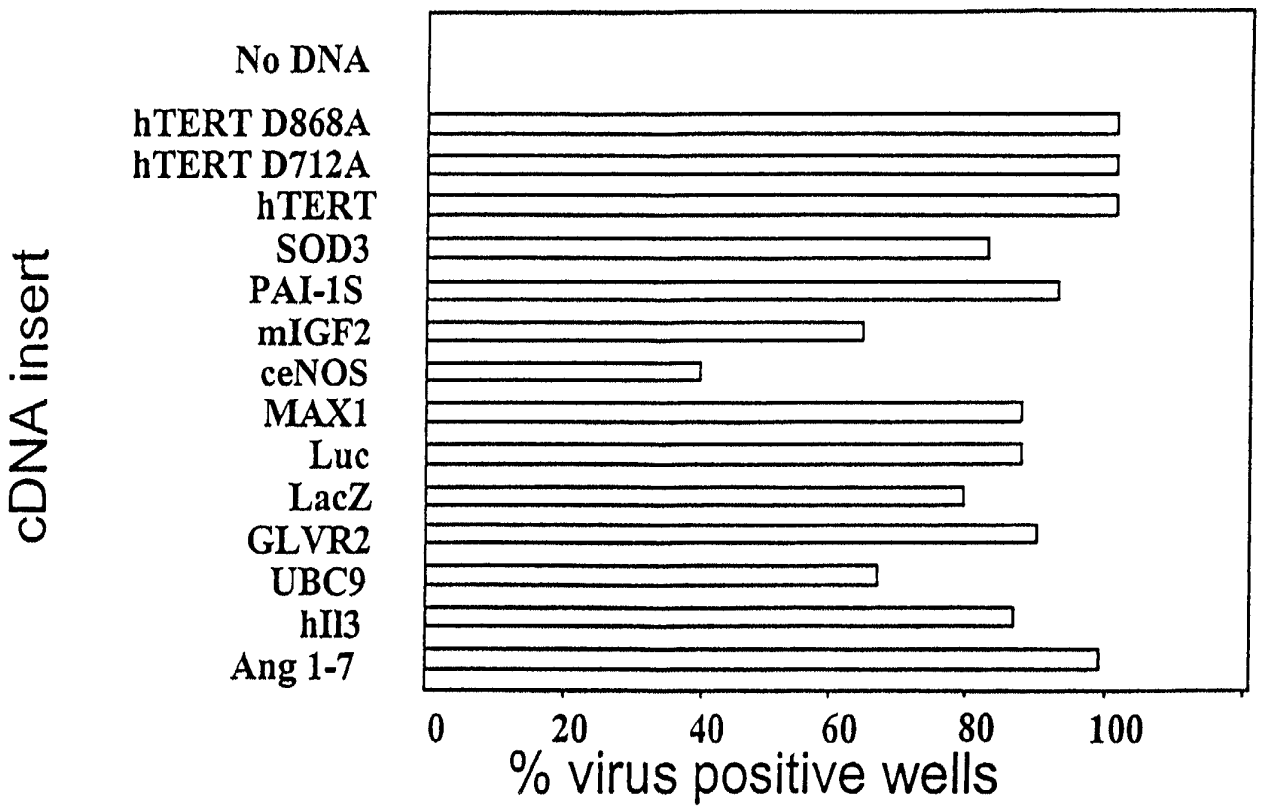


FIG. 26C



Average percentage CPE efficiency: 86 %

FIG. 27

Gene	Insert kb
• ceNOS	3.6
• hTERT	3.5
• hTERT D712A	3.5
• lacZ	3.2
• hCAT1	2.2
• GLVR2	2.0
• Luc	1.7
• SOD3	1.4
• MAX1	.550
• hVEGF121	.511
• hIL3	.434
• UBC9	.412
• ANG1-7	.104

Average titer
$0.8 \pm 0.7 \times 10^9$ pfu/ml

FIG. 28

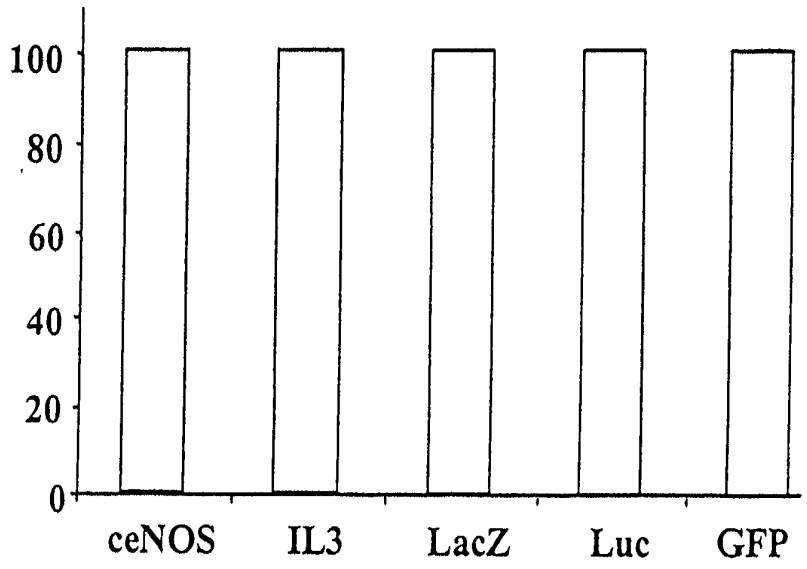


FIG. 29

Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

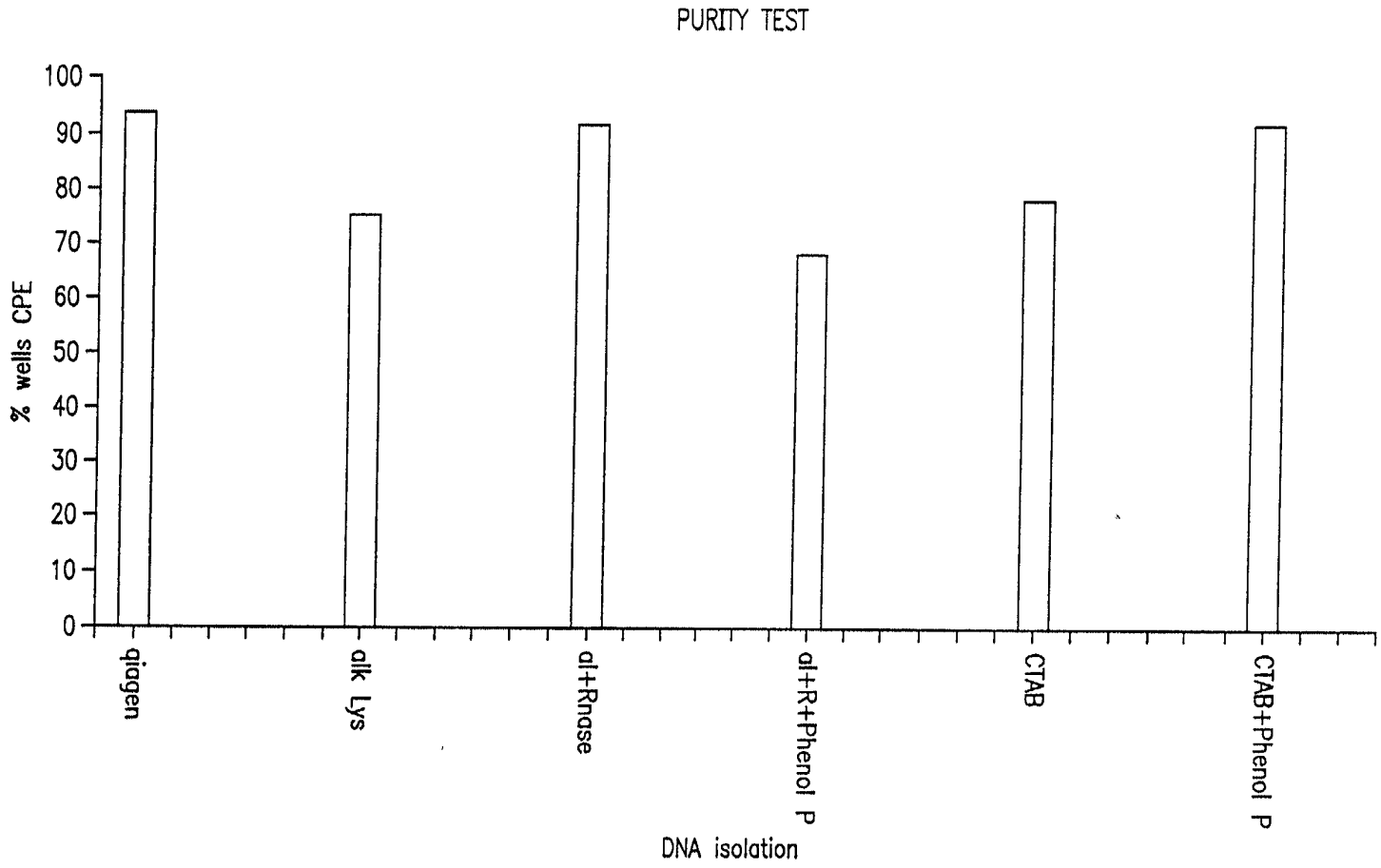


FIG. 30

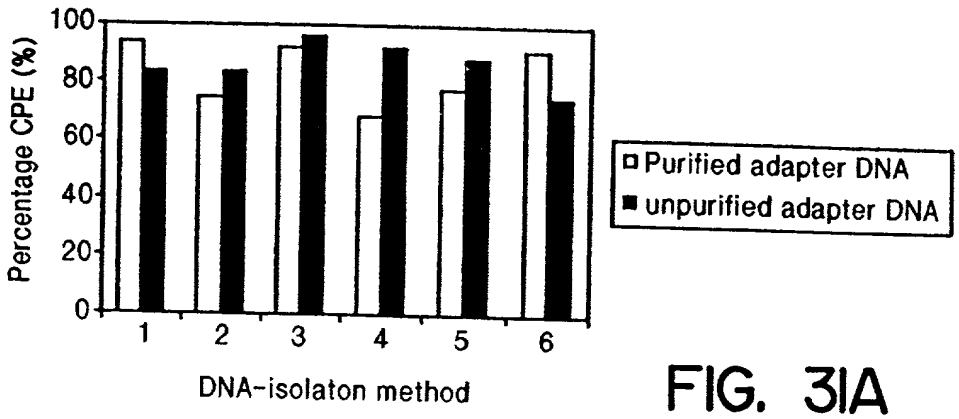


FIG. 31A

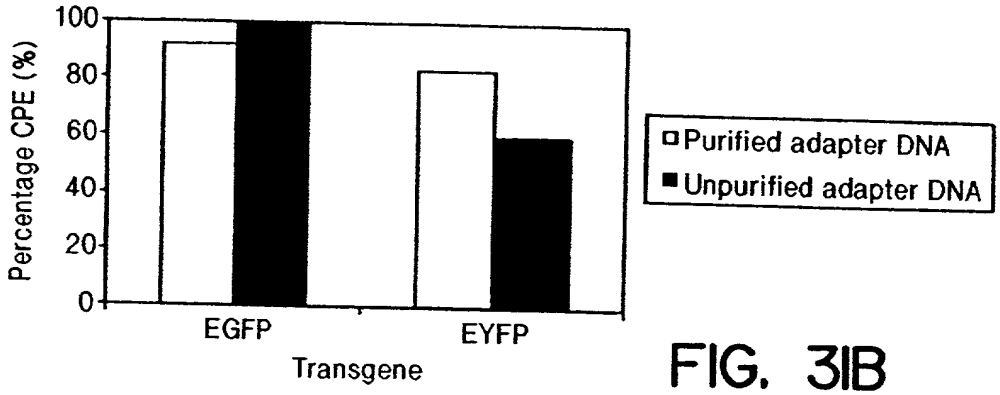


FIG. 31B

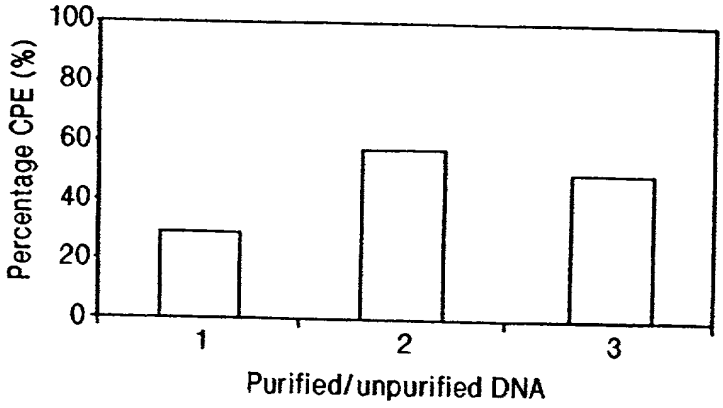
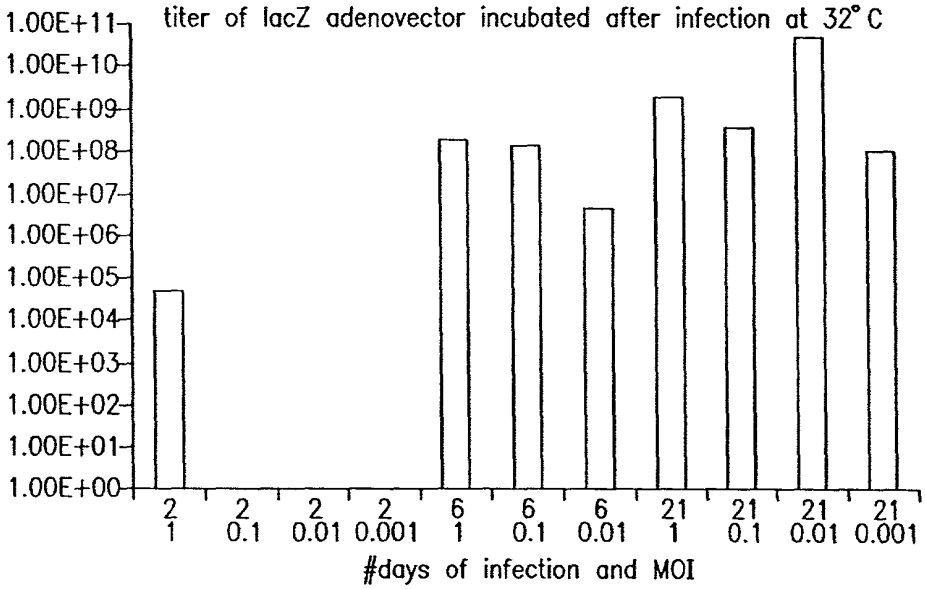
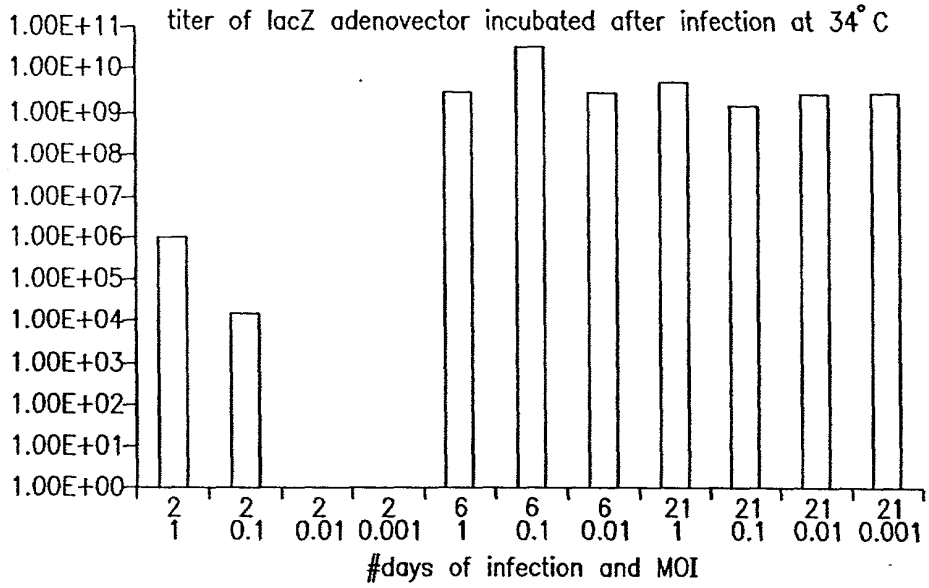


FIG. 31C


titer of lacZ adenovector incubated after infection at 32° C
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
2 1
2 0.1
2 0.01
2 0.001
6 1
6 0.1
6 0.01
21 1
21 0.1
21 0.01
21 0.001
#days of infection and MOI


titer of lacZ adenovector incubated after infection at 34° C
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
2 1
2 0.1
2 0.01
2 0.001
6 1
6 0.1
6 0.01
21 1
21 0.1
21 0.01
21 0.001
#days of infection and MOI

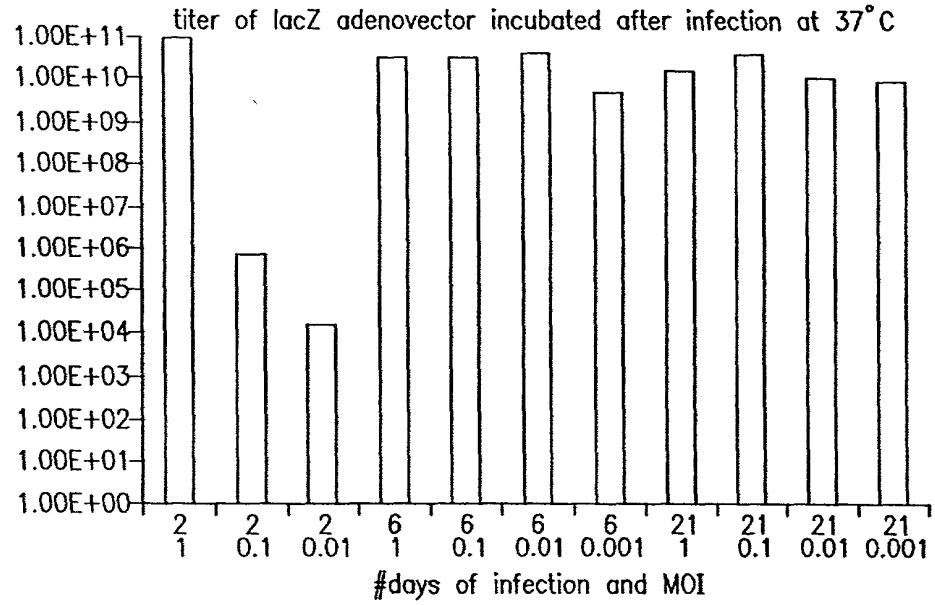

titer of lacZ adenovector incubated after infection at 37° C
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
2 1
2 0.1
2 0.01
6 1
6 0.1
6 0.01
6 0.001
21 1
21 0.1
21 0.01
21 0.001
#days of infection and MOI

FIG. 32

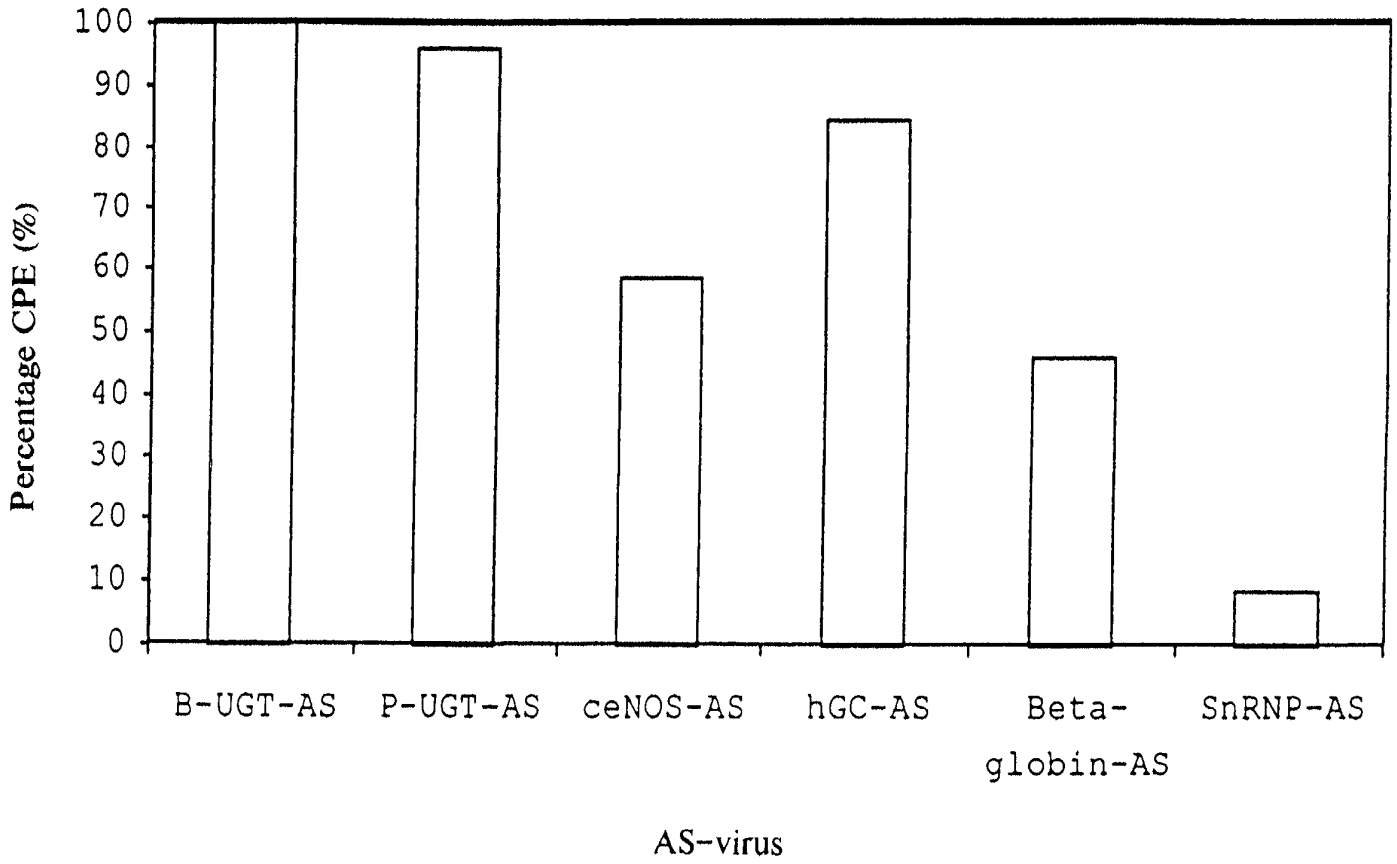


FIG. 33

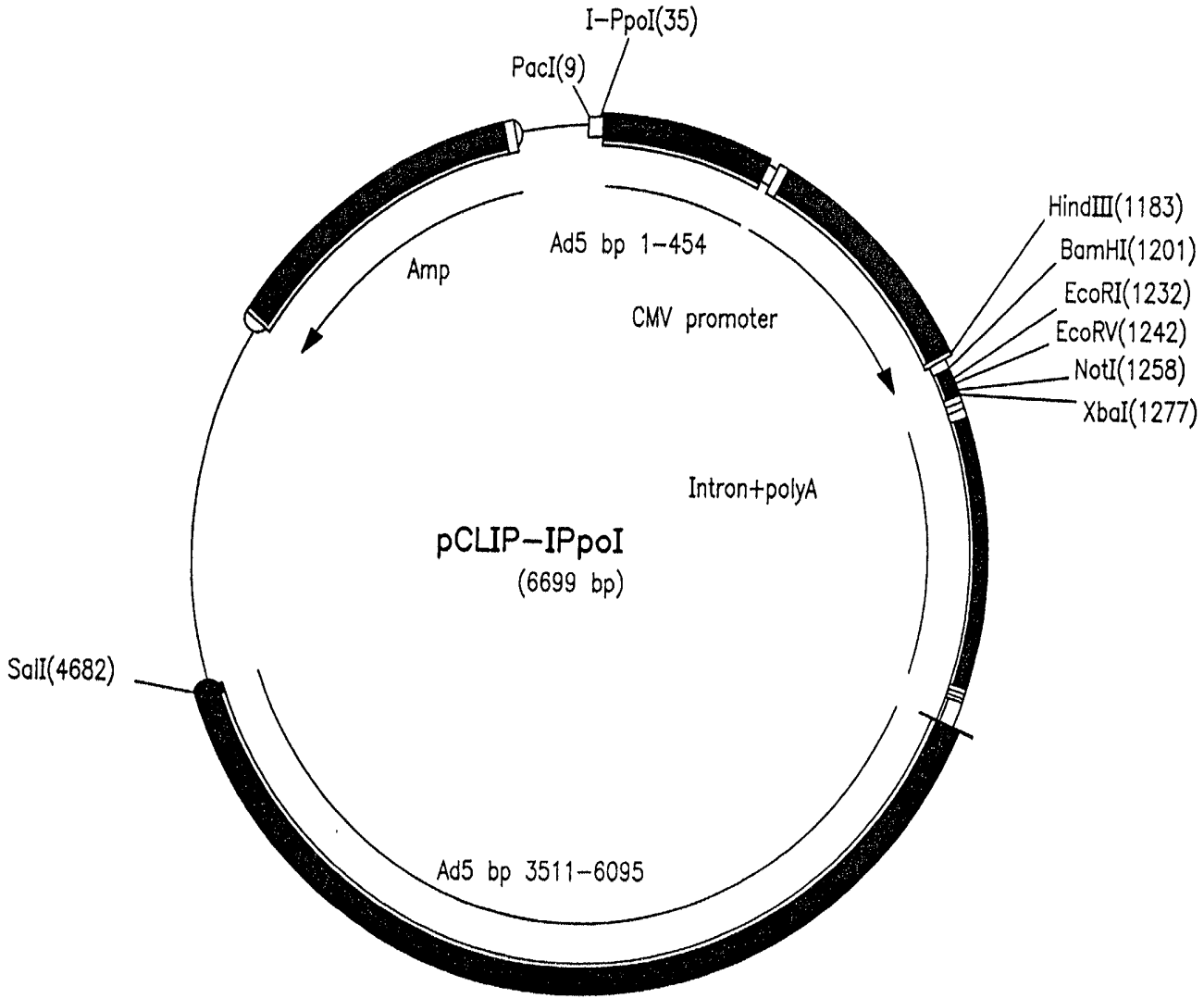

I-PpoI(35)
PacI(9)
HindIII(1183)
BamHI(1201)
EcoRI(1232)
EcoRV(1242)
NotI(1258)
XbaI(1277)
Ad5 bp 1-454
Amp
CMV promoter
Intron+polyA
pCLIP-IPpoI
(6699 bp)
SalI(4682)
Ad5 bp 3511-6095

FIG. 34A

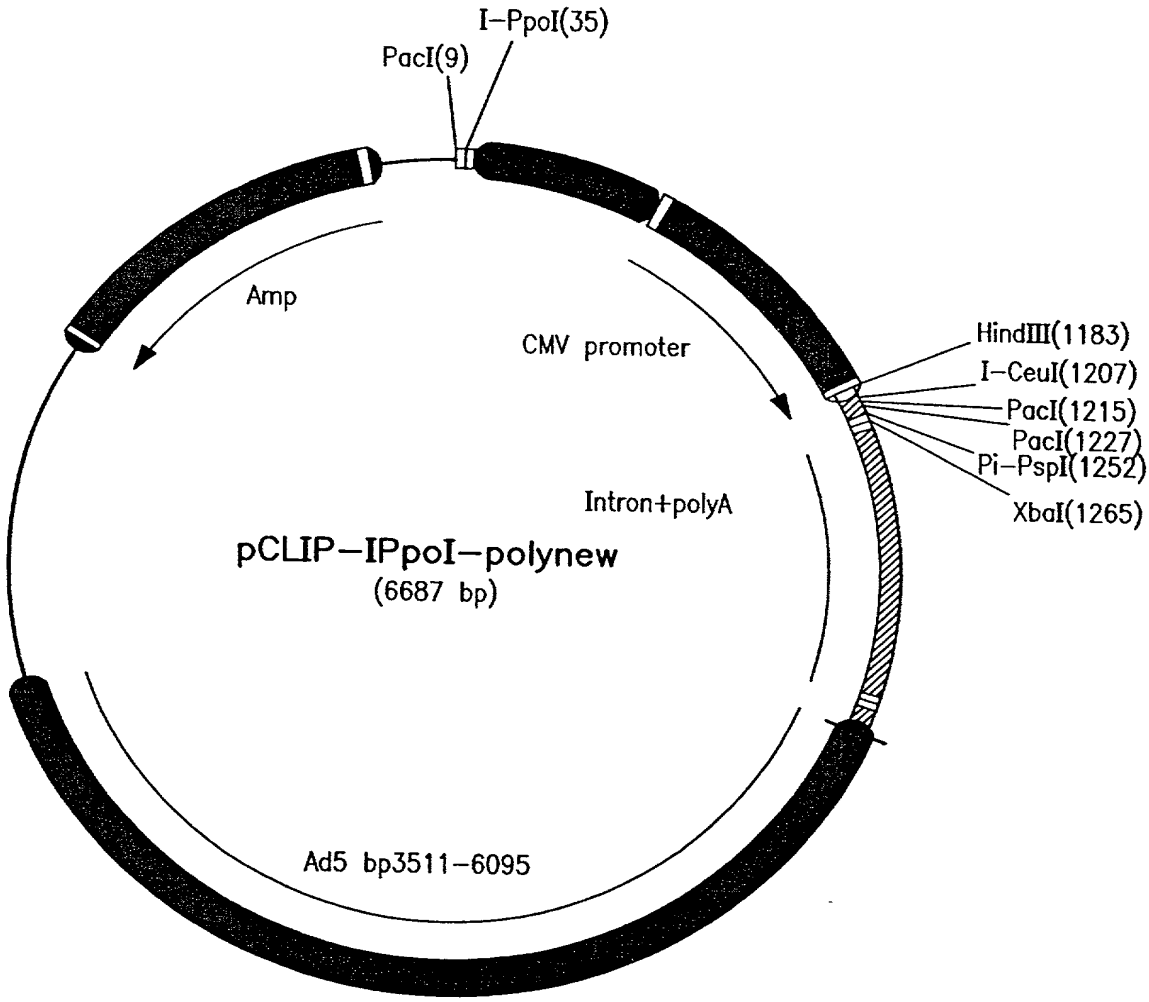

I-PpoI(35)
PacI(9)
Amp
CMV promoter
HindIII(1183)
I-CeuI(1207)
PacI(1215)
PacI(1227)
Pi-PspI(1252)
XbaI(1265)
Intron+polyA
pCLIP-IPpoI-polynew
(6687 bp)
Ad5 bp3511-6095

FIG. 34B

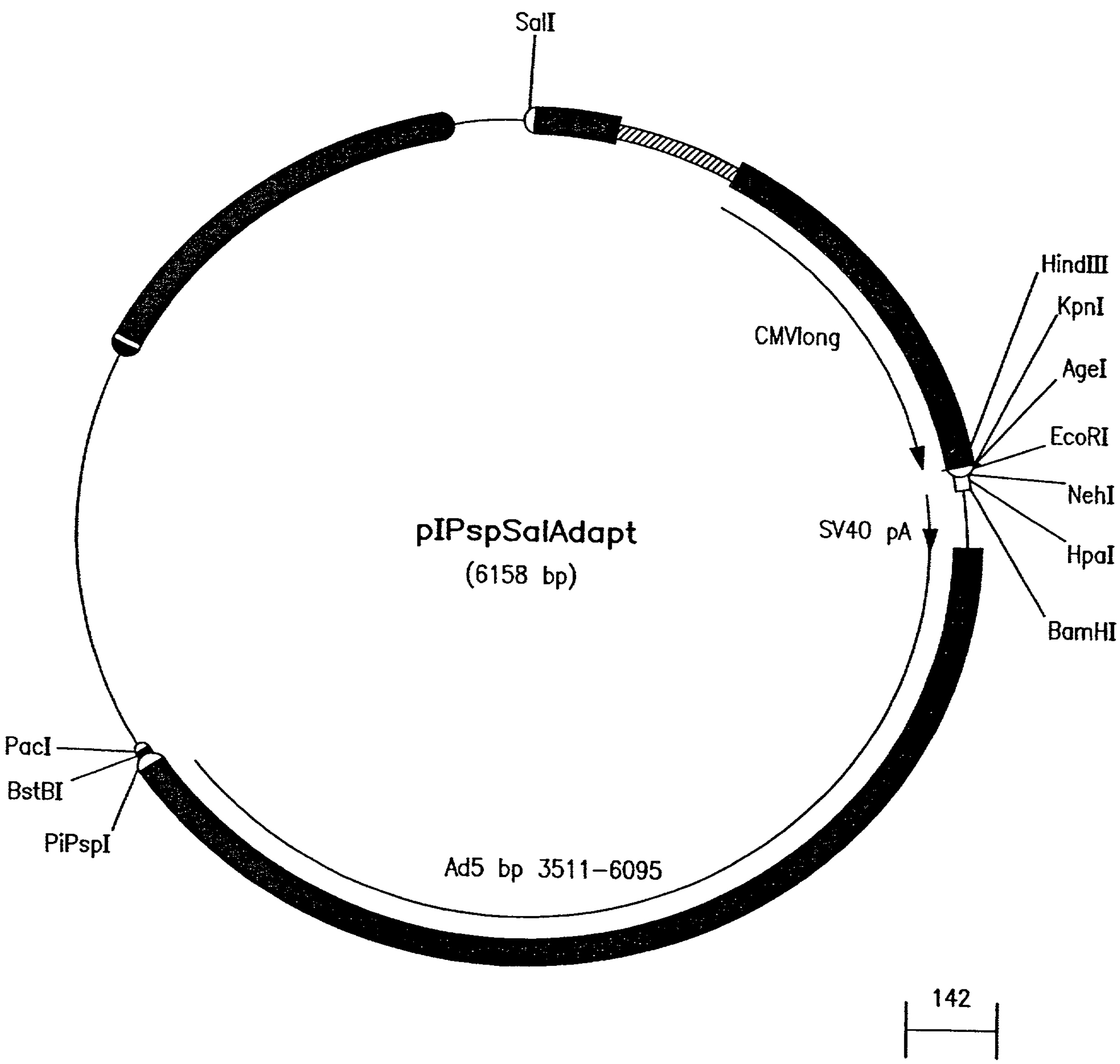

SalI
HindIII
KpnI
AgeI
EcoRI
NehI
HpaI
BamHI
CMVlong
SV40 pA
pIPspSalAdapt
(6158 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095
142

FIG. 34C

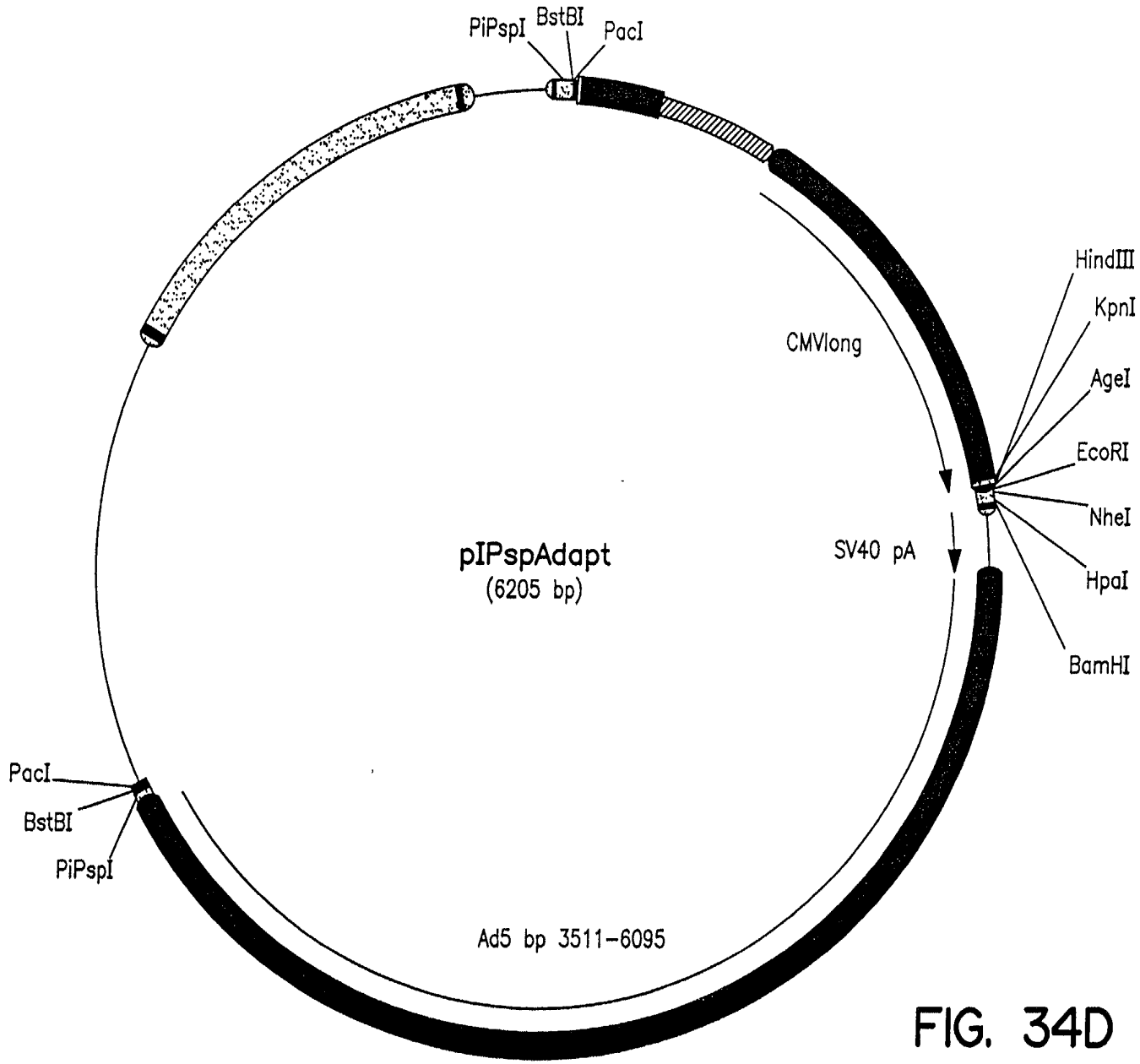


FIG. 34D

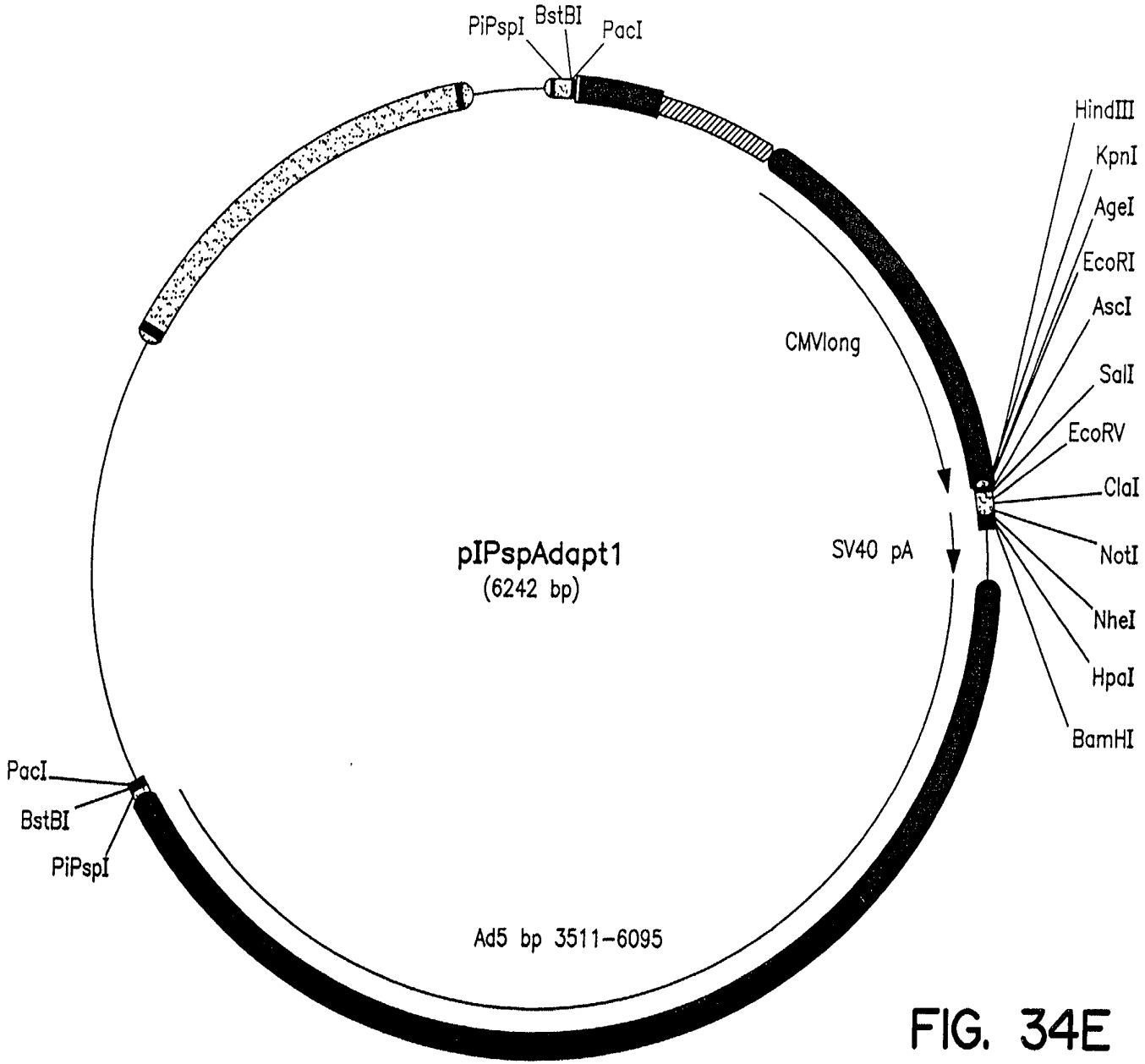


FIG. 34E

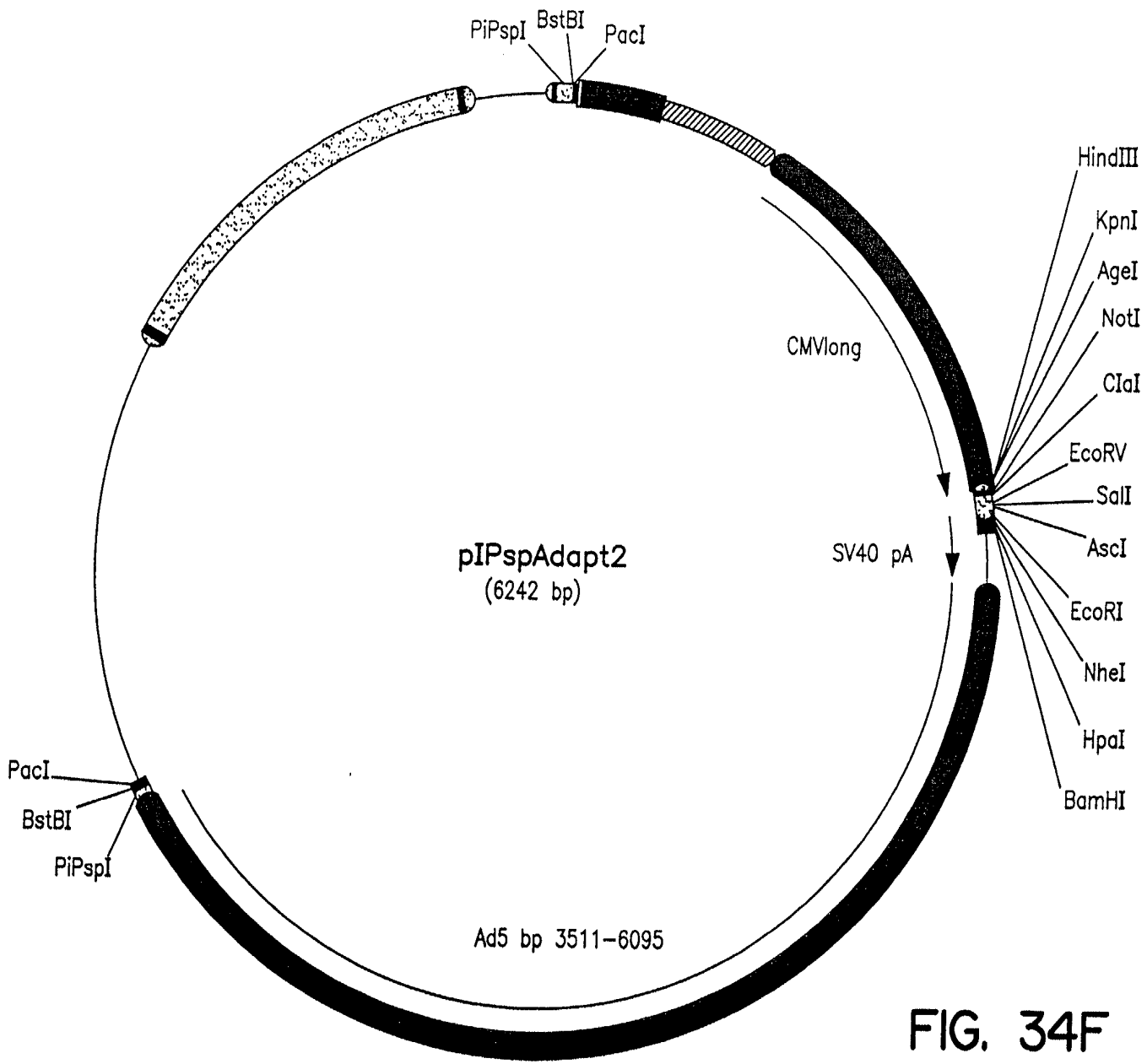


FIG. 34F

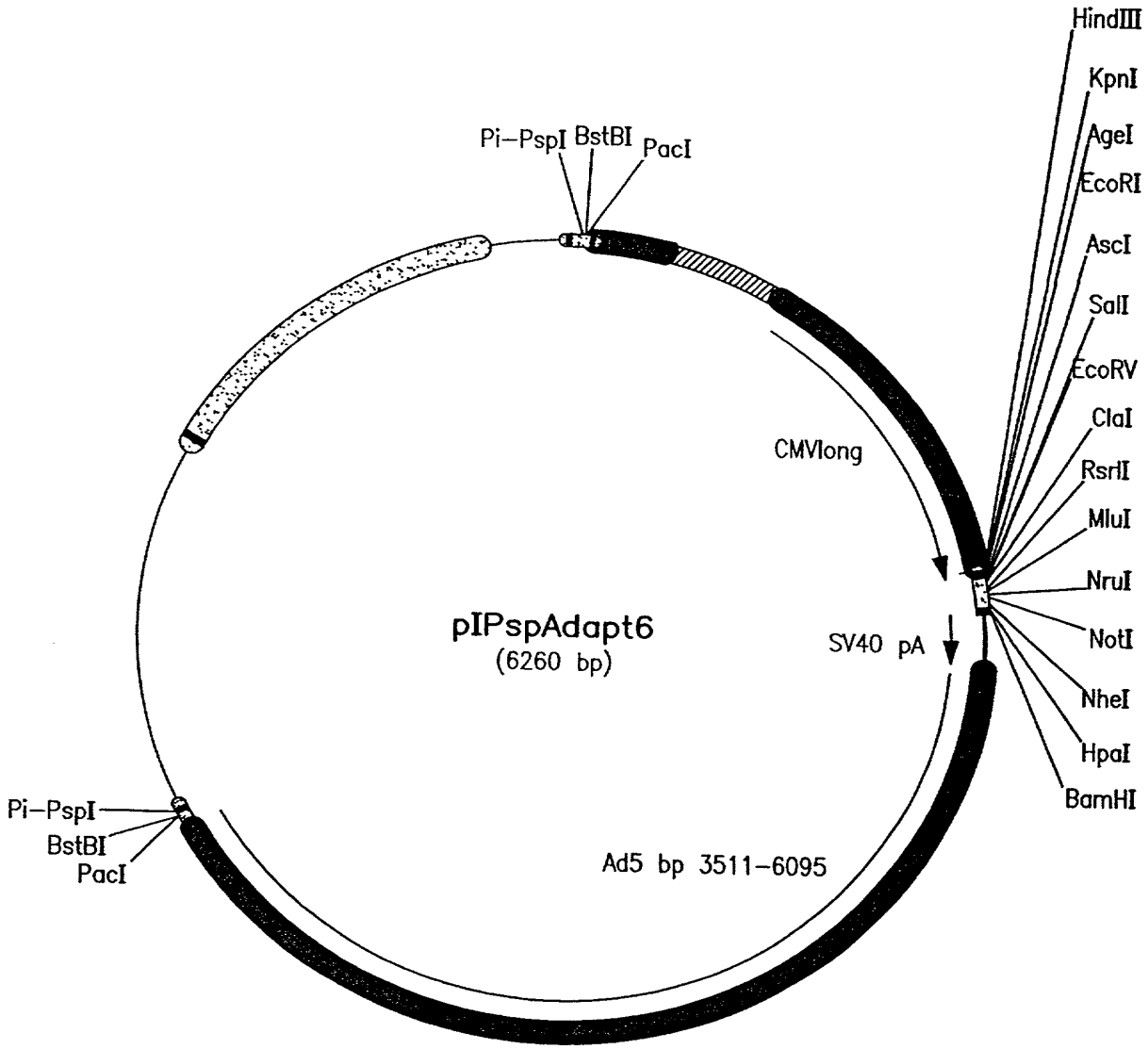

HindIII
KpnI
AgeI
EcoRI
AscI
SalI
EcoRV
ClaI
RsrII
MluI
NruI
NotI
NheI
HpaI
BamHI
Pi–PspI
BstBI
PacI
CMVlong
pIPspAdapt6
(6260 bp)
SV40 pA
Pi–PspI
BstBI
PacI
Ad5 bp 3511–6095

FIG. 34G

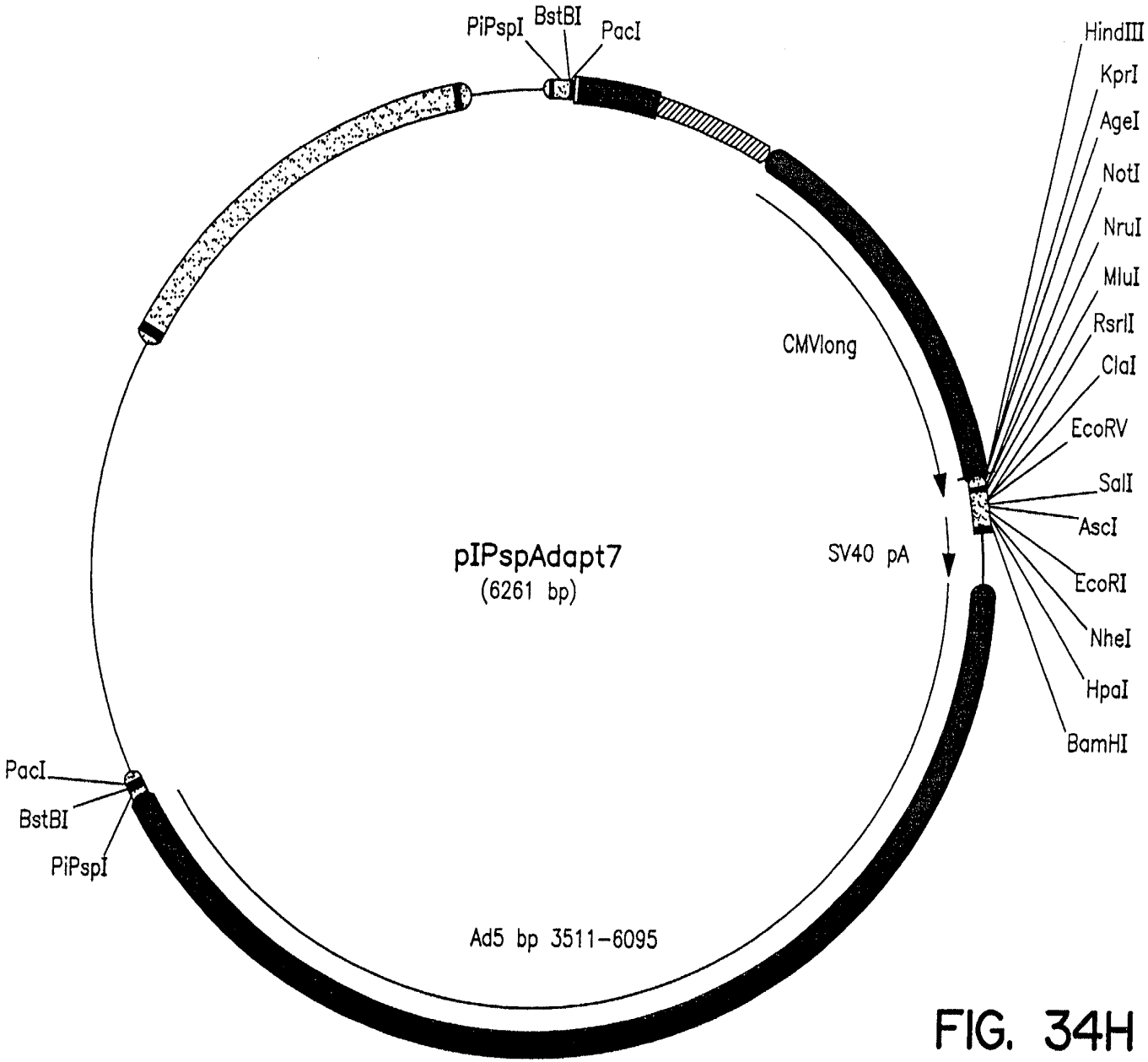


FIG. 34H

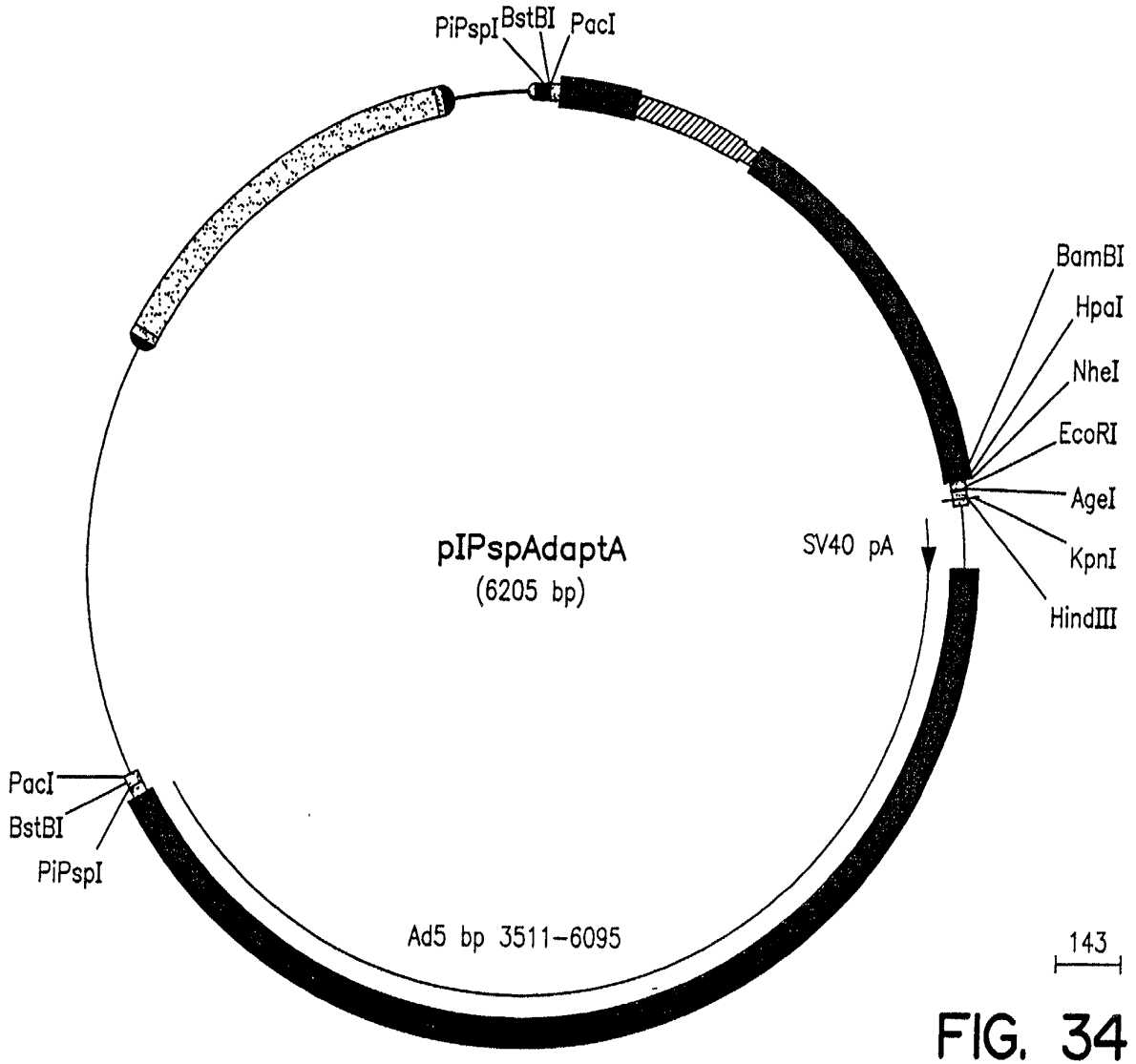


FIG. 34 I

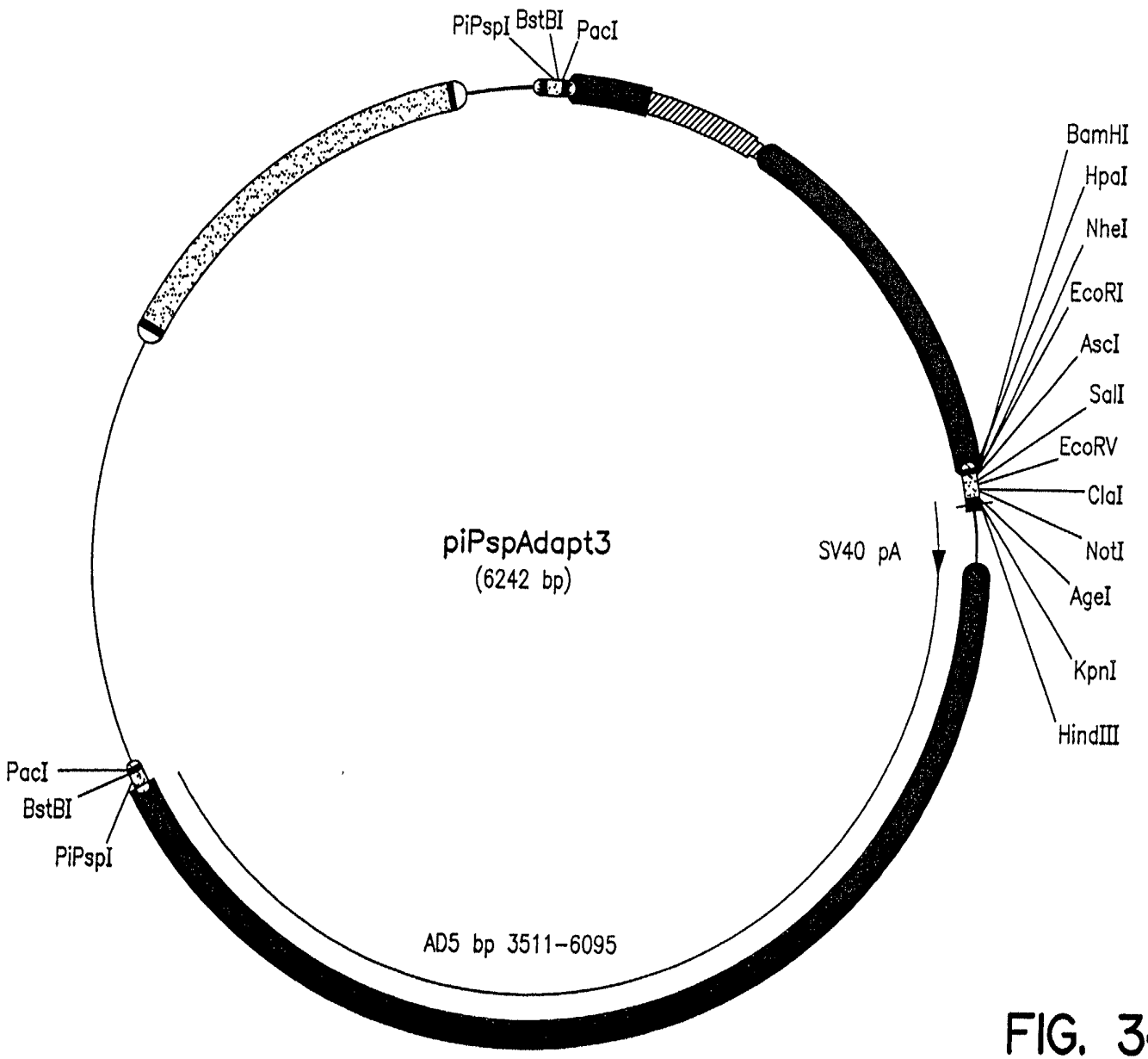


FIG. 34J

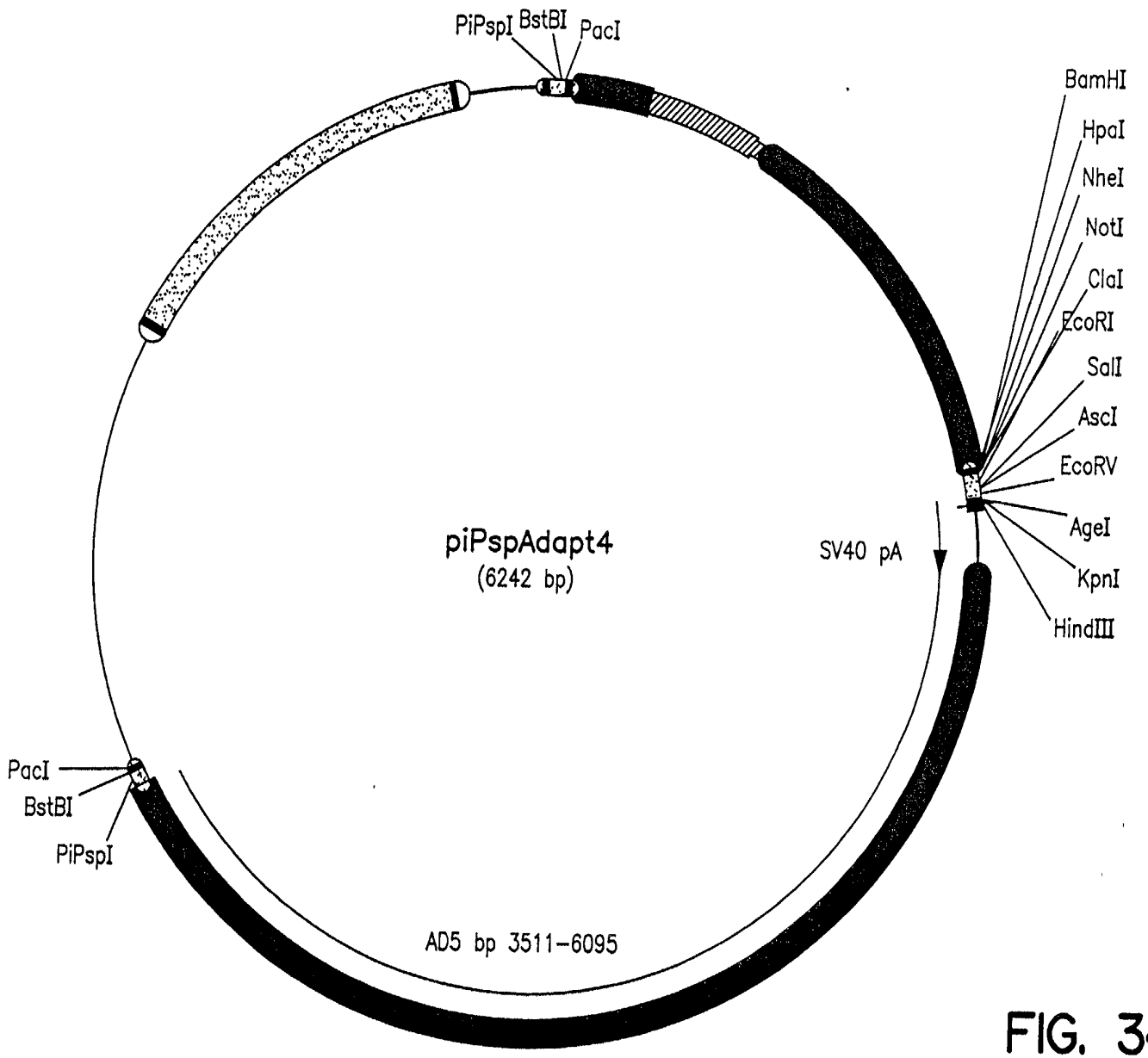


FIG. 34K

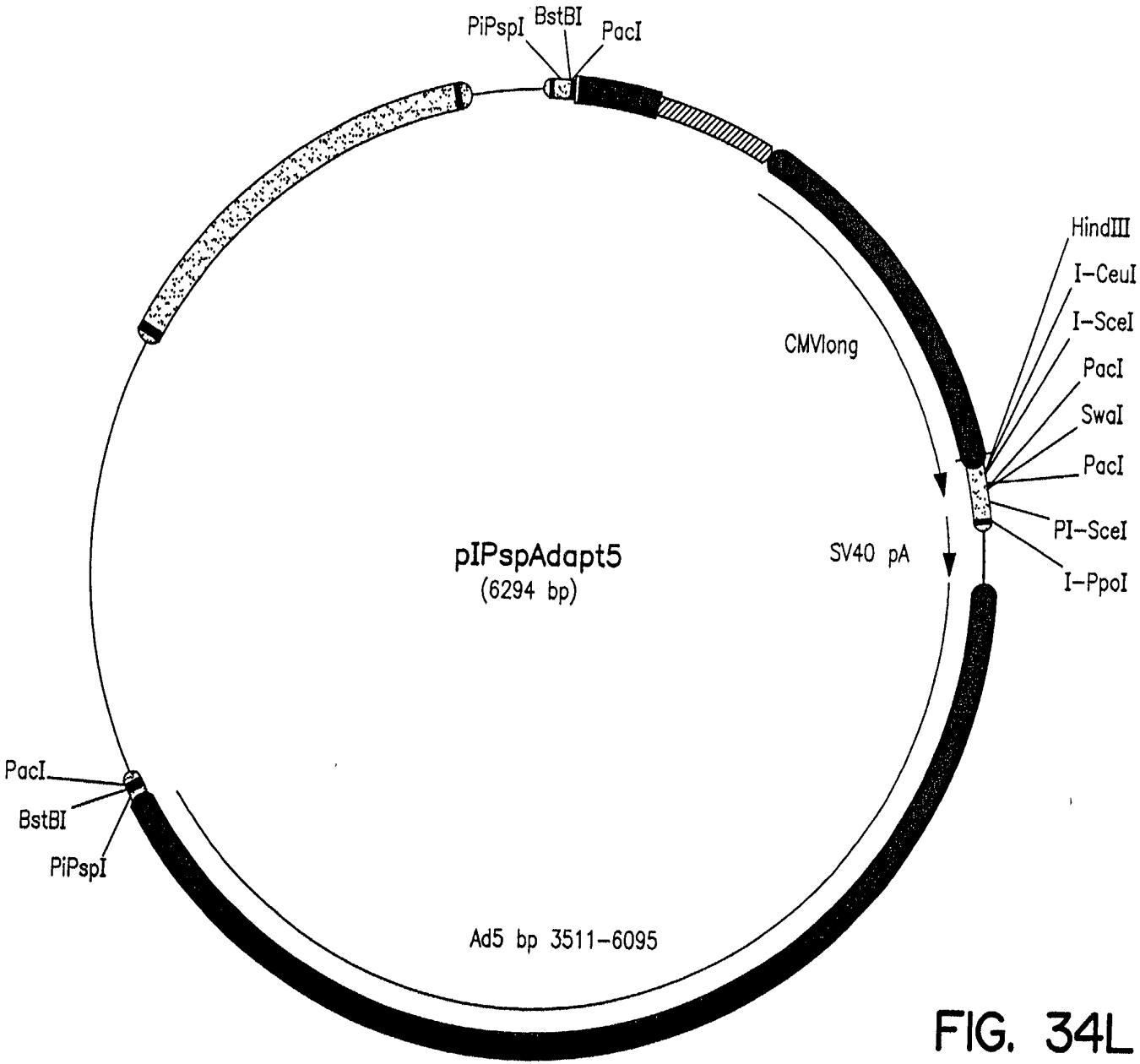


FIG. 34L

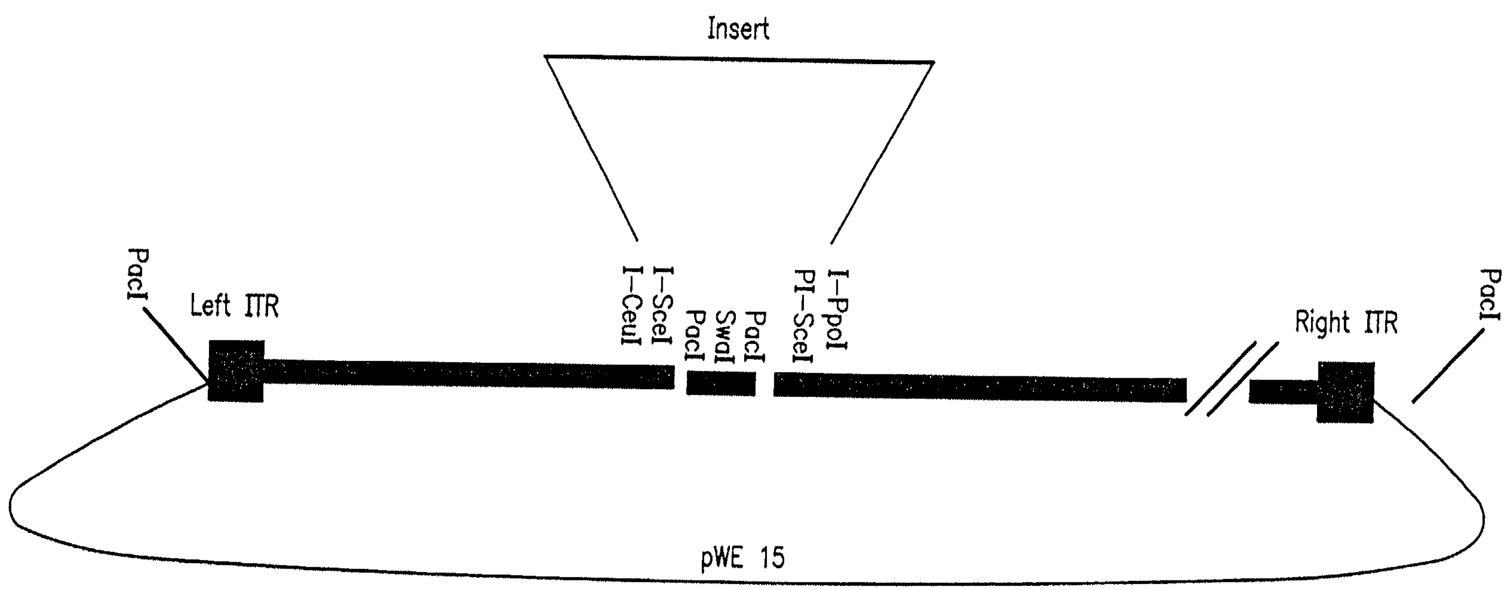

Insert
PacI
Left ITR
I-SceI
I-CeuI
PacI
SwaI
PacI
I-PpoI
PI-SceI
Right ITR
PacI
pWE 15

FIG. 34M

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP–LacZ and the adapter plasmid pIPspAdapt2.

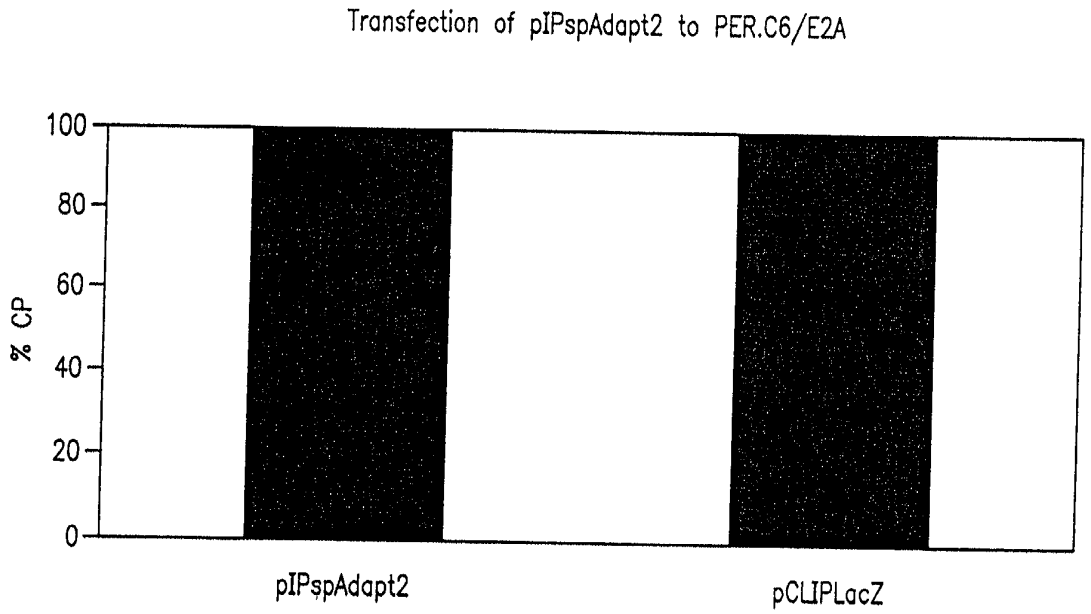


FIG. 34N

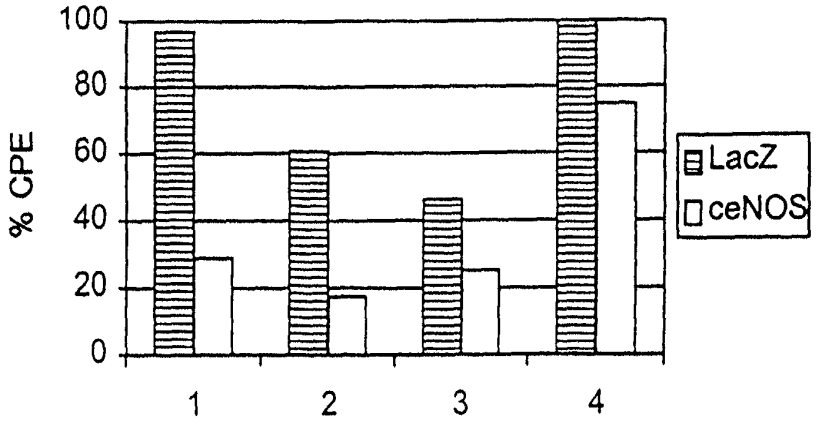


FIG. 35

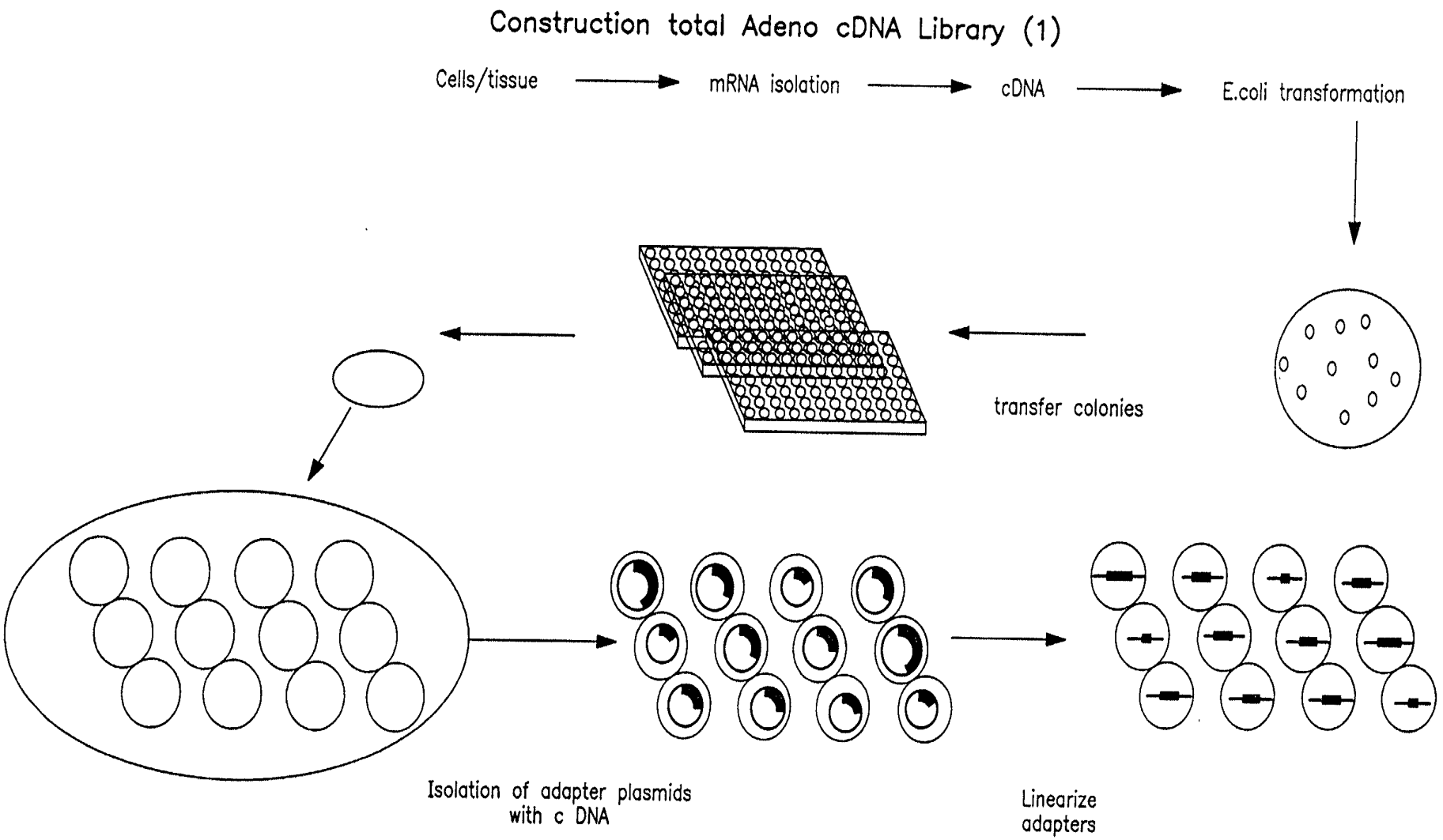


FIG. 36A

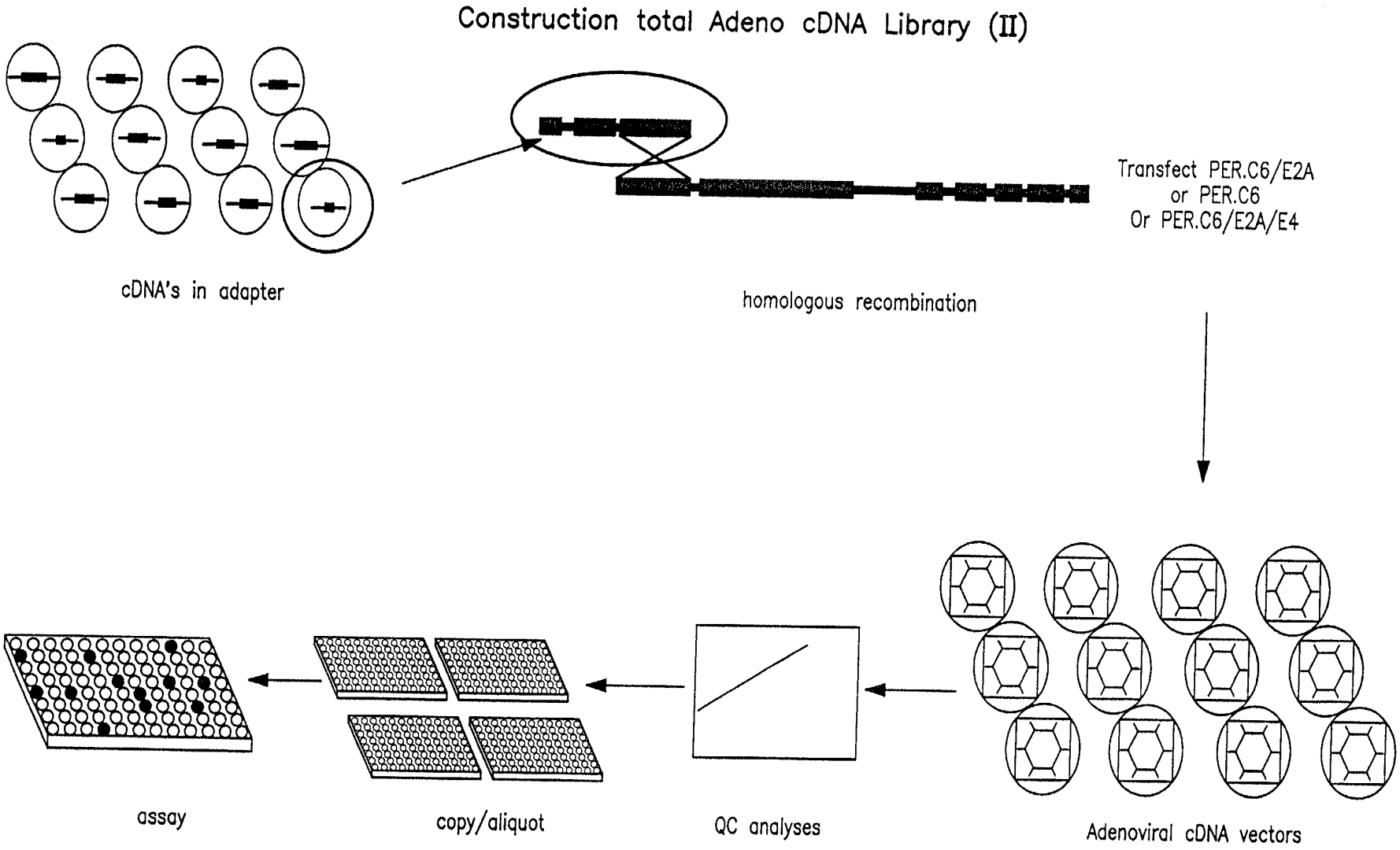


FIG. 36B

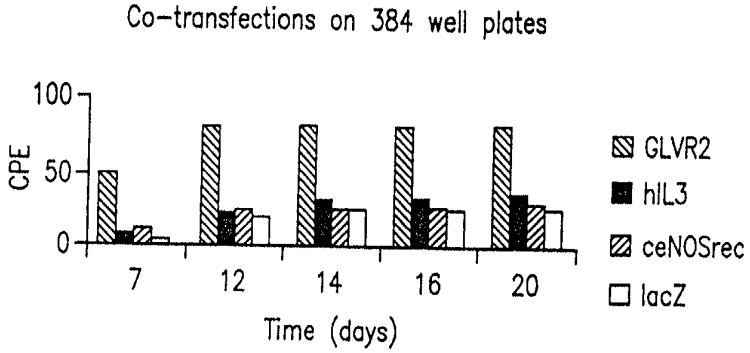


FIG. 37A

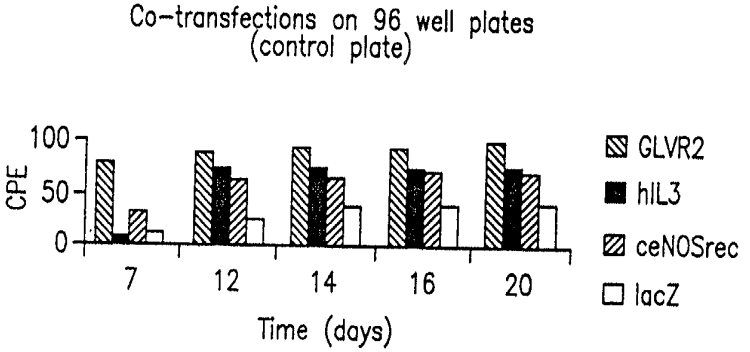


FIG. 37B

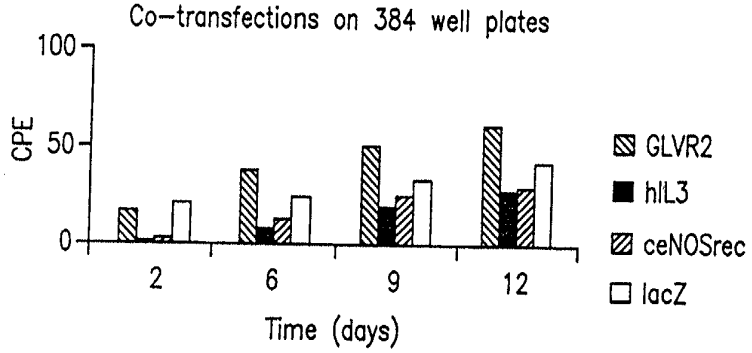


FIG. 37C

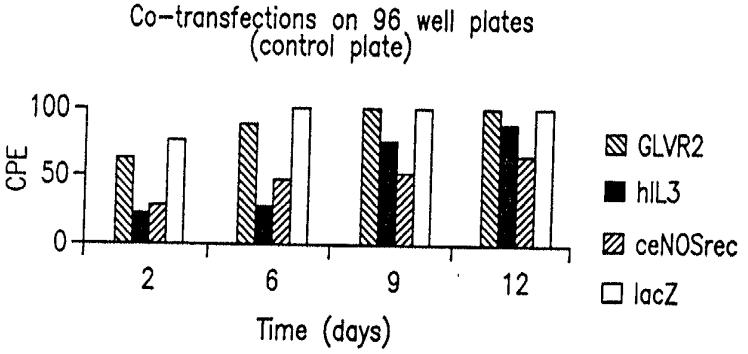


FIG. 37D

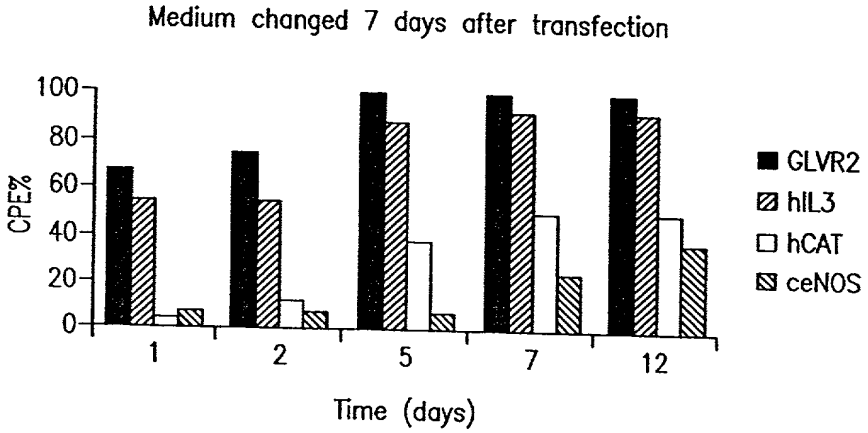


FIG. 38A

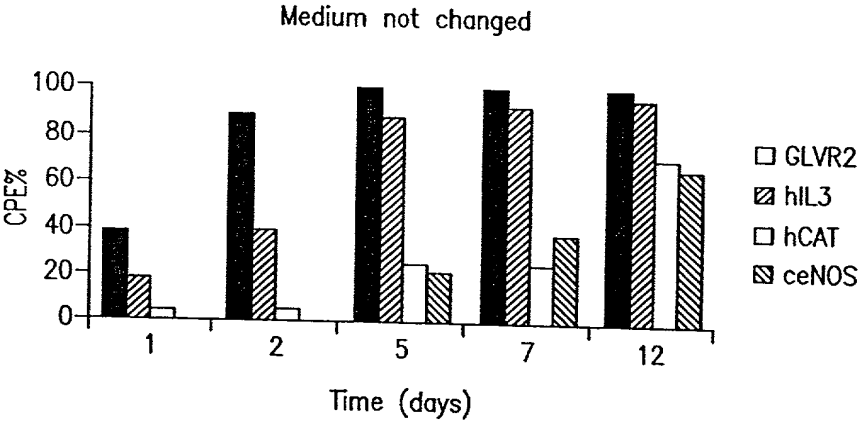


FIG. 38B

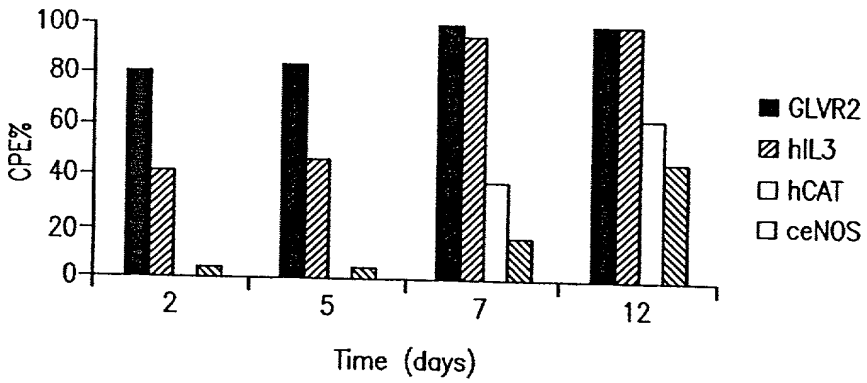


FIG. 38C

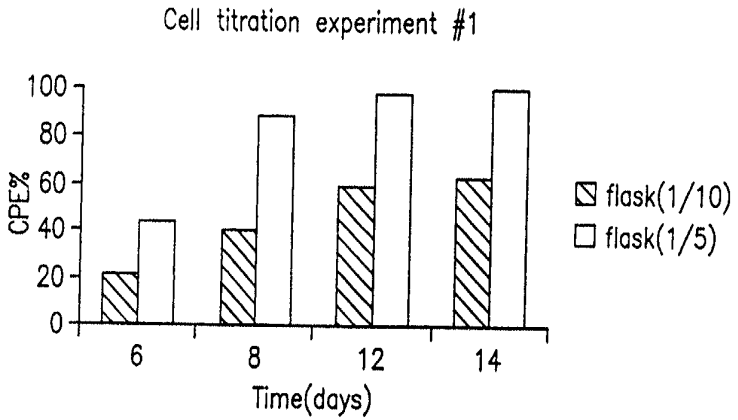


FIG. 39A

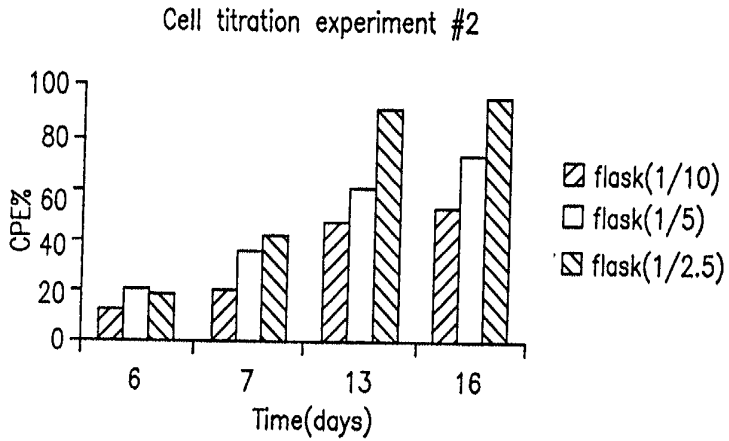


FIG. 39B

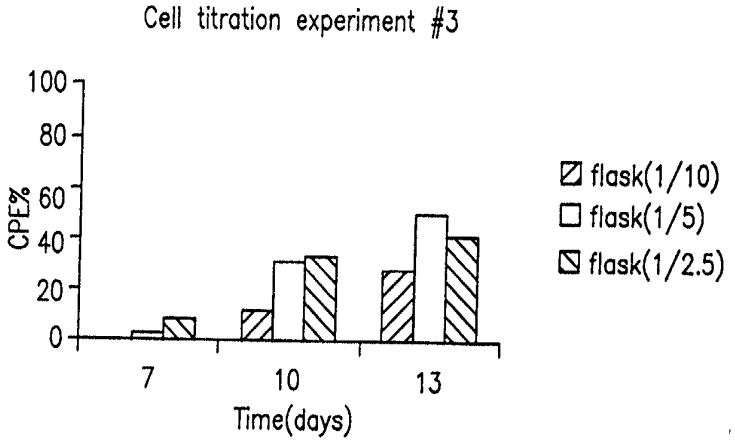


FIG. 39C

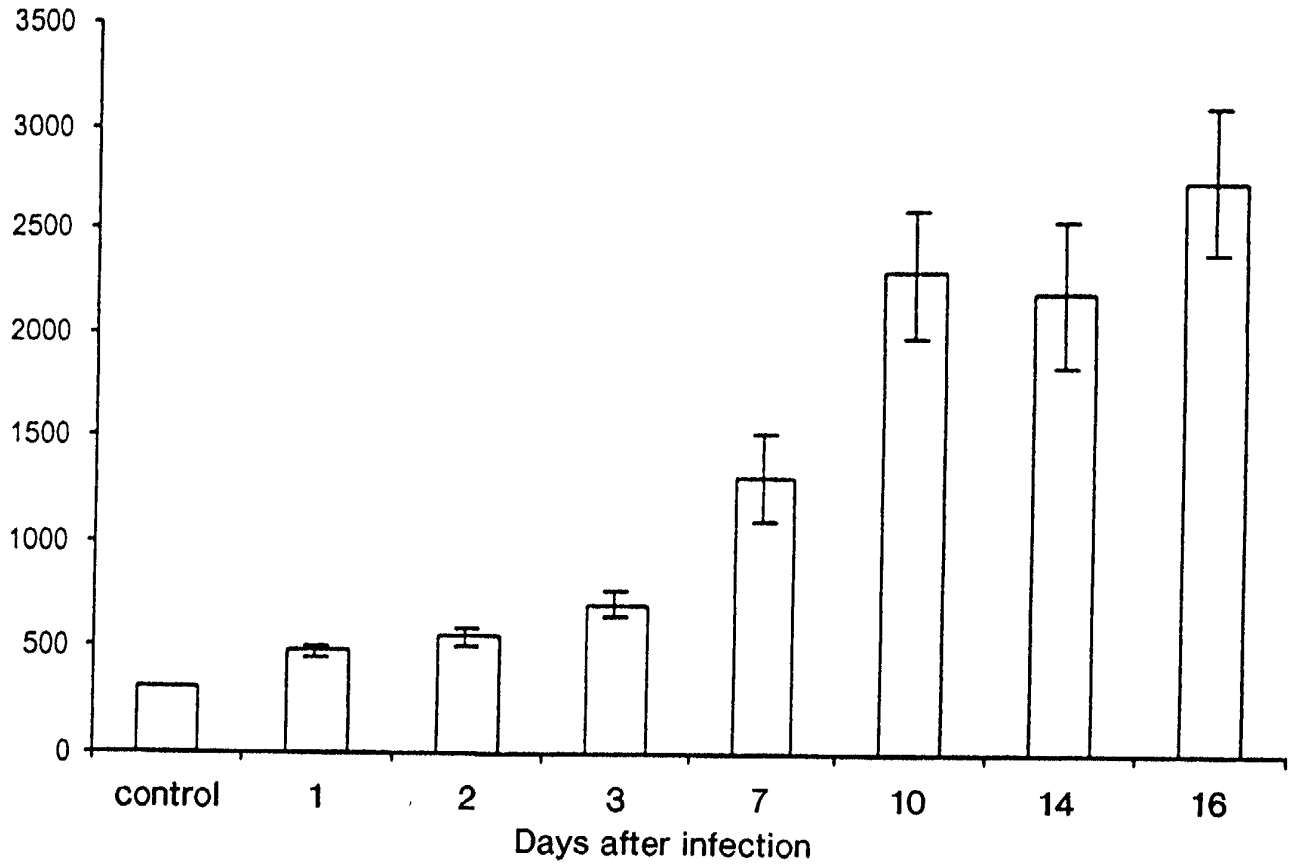


FIG. 40

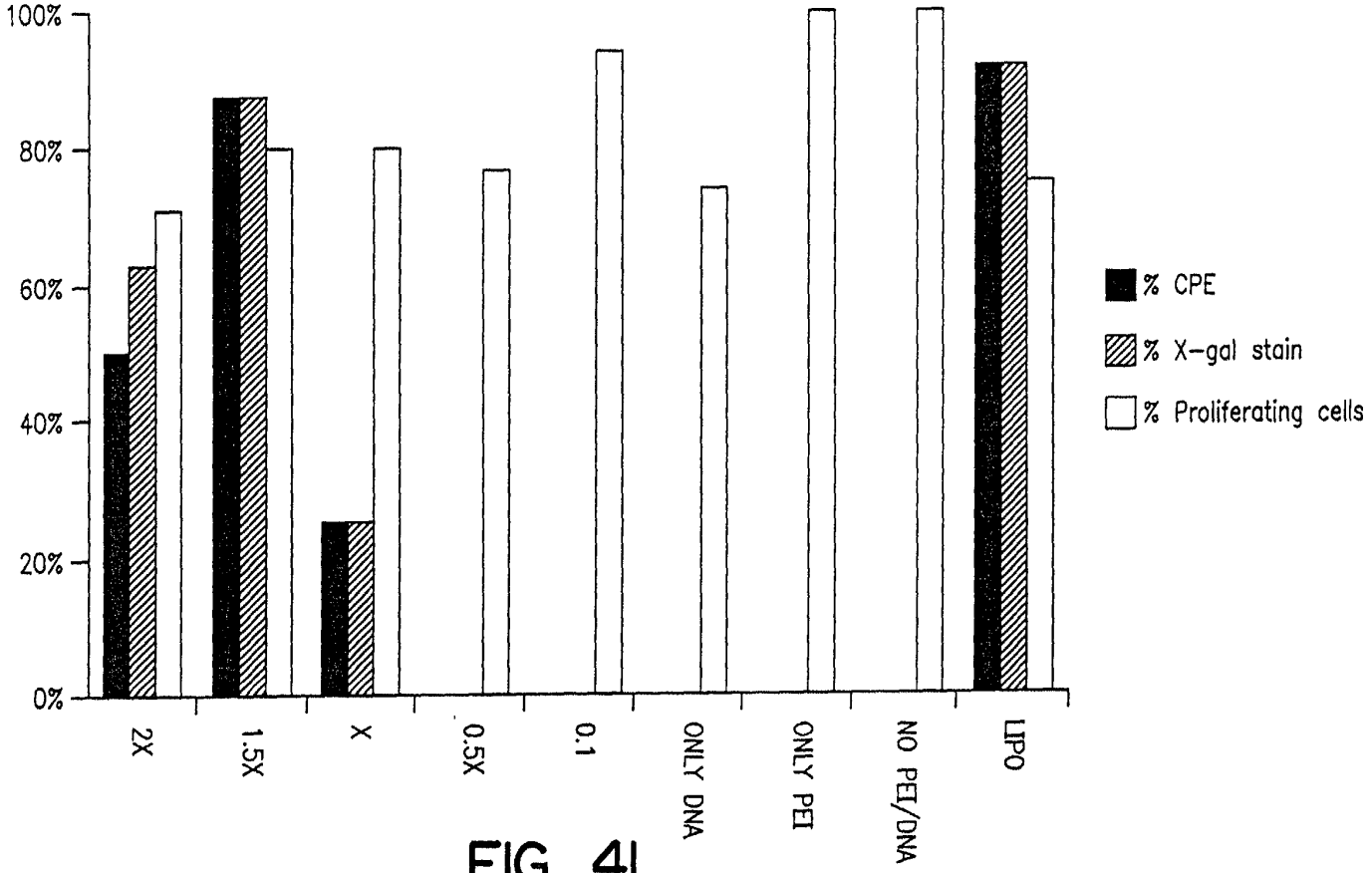


FIG. 41

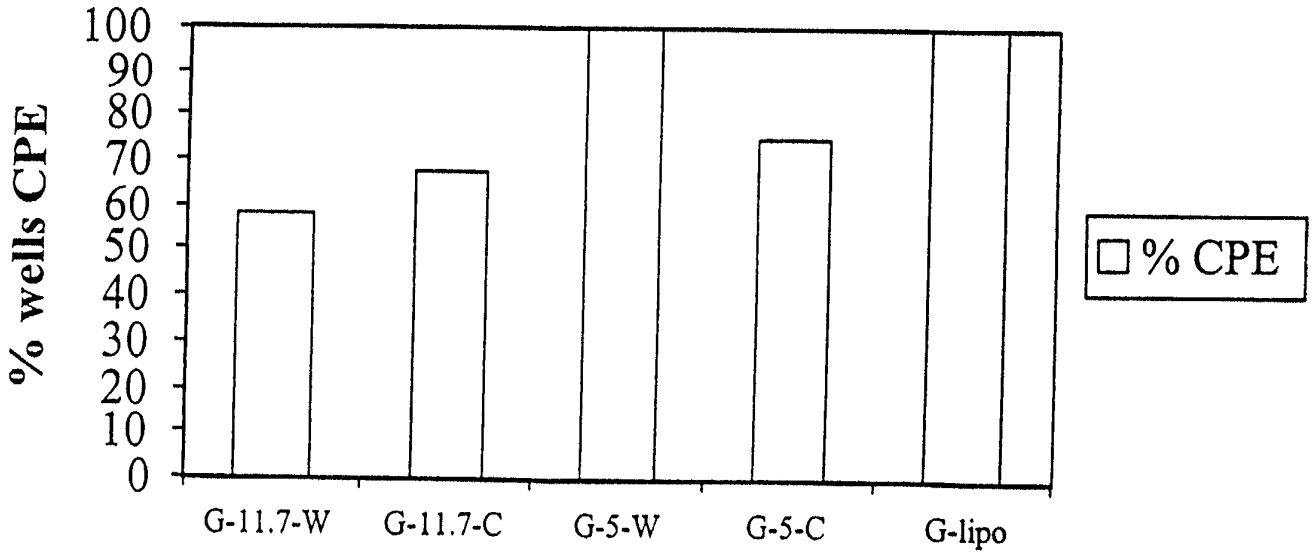


FIG. 42

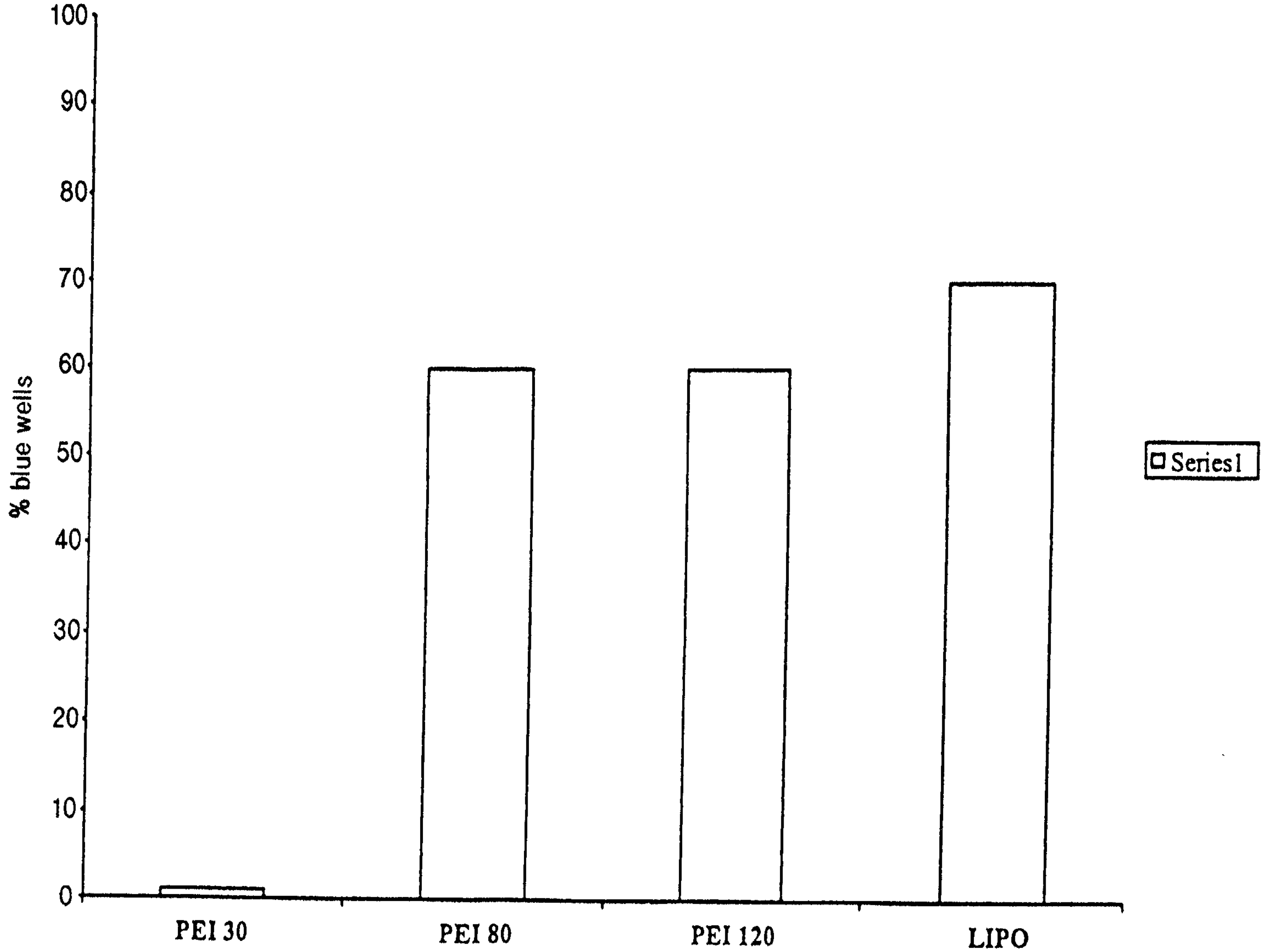

100
90
80
70
60
50
40
30
20
10
0
% blue wells
Series1
PEI 30
PEI 80
PEI 120
LIPO

FIG. 43

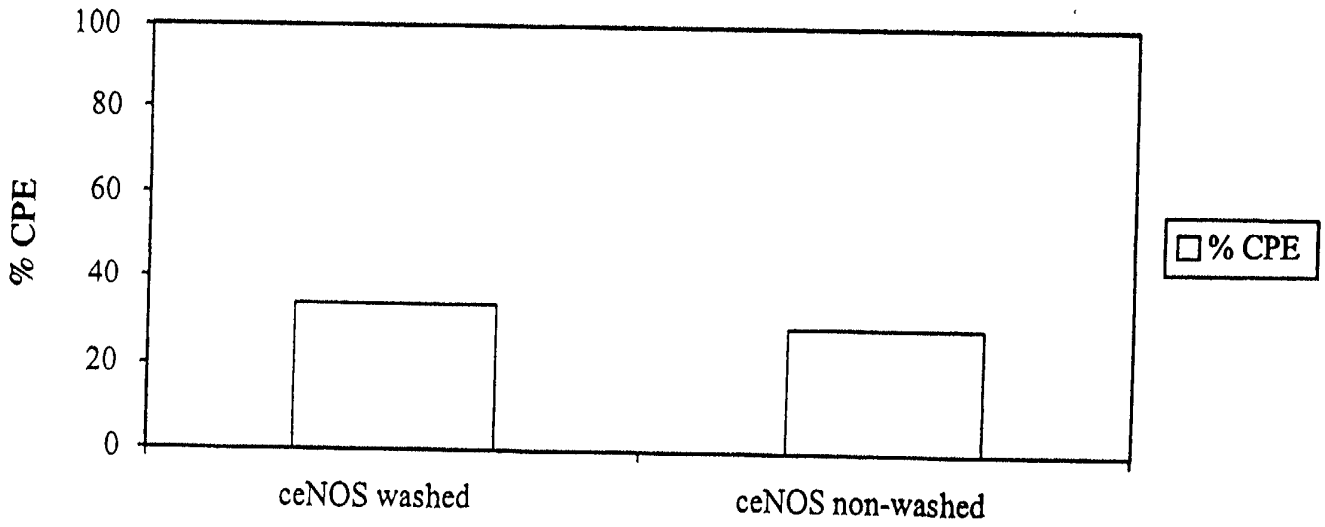


FIG. 44